

Louisiana State University LSU Digital Commons

LSU Doctoral Dissertations

Graduate School

2003

Effects of submergence and hypoxia on the growth and anatomy of rice (*Oryza sativa* L.) seedlings

Denise M. D'Abundo

Louisiana State University and Agricultural and Mechanical College

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_dissertations

Recommended Citation

D'Abundo, Denise M., "Effects of submergence and hypoxia on the growth and anatomy of rice (*Oryza sativa* L.) seedlings" (2003).
LSU Doctoral Dissertations. 1952.
https://digitalcommons.lsu.edu/gradschool_dissertations/1952

This Dissertation is brought to you for free and open access by the Graduate School at LSU Digital Commons. It has been accepted for inclusion in LSU Doctoral Dissertations by an authorized graduate school editor of LSU Digital Commons. For more information, please contact gradetd@lsu.edu.

**EFFECTS OF SUBMERGENCE AND HYPOXIA ON THE GROWTH AND ANATOMY
OF RICE (*ORYZA SATIVA* L.) SEEDLINGS**

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Plant Biology

by
Denise M. D'Abundo
B.A., Oberlin College, 1992
May 2003

©Copyright 2003
Denise Marie D'Abundo
All rights reserved

DEDICATION

I dedicate this dissertation to my parents, Anthony and Carol D'Abundo. Their unconditional, multifaceted and loving support made earning this degree possible.

ACKNOWLEDGEMENTS

I gratefully acknowledge my advisor, Dr. Dave Longstreth, for providing unobtrusive yet enduring guidance. To my committee members, Dr. Karen McKee, Dr. Irv Mendelssohn and Dr. Jim Moroney and Dean's representative, Dr. Jim Oard, I extend my gratitude for their participation and helpful suggestions. Dr. Olga Borkhseniuous and Ying Xiao carried out preliminary microscopy work that stimulated research presented in this Dissertation. Dr. Holly Hale-Donze sincerely tried to help simplify the quantitative analysis of microscopic images. Dr. Marc Cohn and Dr. Chuck Rush generously provided the rice grain for this study. Computer-related assistance provided by Dr. Sergio Columbo was crucial to assembling the dissertation. The comradeship of Dr. Beth Schussler, whose dissertation research brought aerenchyma development to the Longstreth lab, continues to be appreciated. With cherished friends and fellow graduate students, Soon-to-be-Dr. Sandra Guzman, Soon-to-be-Dr. Teresa Venezia and Dr. Jim Pahl, I discovered the motivating answer to why the chicken crossed the road. Shannon Cox, Dr. Dorothy Hernandez and Cheryl Schairer have been supportive, inspirational friends to me before, during, and after this arduous metamorphosis. My parents, Carol and Anthony D'Abundo, my sister, Michelle D'Abundo, and my extended family members offered wholehearted encouragement without ever making me feel as if an additional degree would make me a better person. I thank all of these people for helping me get to the point where I can set sights on other finish lines.

TABLE OF CONTENTS

DEDICATION	iii
ACKNOWLEDGEMENTS	iv
LIST OF TABLES	vi
LIST OF FIGURES	vii
ABSTRACT	ix
CHAPTER 1. INTRODUCTION TO STUDY	1
CHAPTER 2. EFFECTS OF SUBMERGENCE AND HYPOXIA ON THE GROWTH OF RICE SEEDLINGS	4
Introduction	4
Materials and Methods	6
Results	12
Discussion	21
CHAPTER 3. EFFECTS OF SUBMERGENCE AND HYPOXIA ON THE ANATOMY OF RICE SEEDLINGS	28
Introduction	28
Materials and Methods	31
Results	33
Discussion	47
CHAPTER 4. SUMMARY OF STUDY	53
LITERATURE CITED	56
APPENDIX 1. GROWTH OF SEEDLINGS USED FOR ANATOMICAL ANALYSIS	63
APPENDIX 2. ROOT RADII AND THICKNESSES OF THE CORTEX, STELE AND OUTER LAYERS	64
VITA	67

LIST OF TABLES

2.1. Mean leaf emergence times (± 1 S.D.) for Lemont and red rice seedlings.	13
2.2. Mesocotyl formation and length in Lemont and red rice seedlings grown in aerated and hypoxic water.	20
3.1. Mean final lengths (± 1 S.E.) of primary roots and second leaves of Lemont and red rice seedlings grown in aerated and hypoxic water.	35

LIST OF FIGURES

2.1. Experimental box in which seedlings were grown.	8
2.2. A composite figure showing scanned images of one aerated Lemont seedling (left) and one aerated red rice seedling (right) at approximately 48, 72, 96 and 120 h after imbibition.	10
2.3. One Lemont seedling (right) and one red rice seedling (left) approximately 216 h after imbibition. Seedlings grew in continuous darkness while positioned 2.5 cm below the surface of hypoxic water.	11
2.4. Leaf and primary root lengths of Lemont (A, B) and red rice seedlings (C, D) grown in aerated water. Germinated seeds were positioned at three depths (1.0, 2.5 and 4.5 cm)	14
2.5. Leaf (A) and primary root (B) lengths of Lemont seedlings grown in hypoxic water. Germinated seeds were positioned at three depths (1.0, 2.5 and 4.5 cm)	15
2.6. Leaf (A) and primary root (B) lengths of red rice seedlings grown in hypoxic water. Germinated seeds were positioned at three depths (1.0, 2.5 and 4.5 cm)	16
2.7. Leaf, coleoptile and root lengths of Lemont and red rice seedlings grown in aerated water. Germinated seeds were positioned 2.5 cm below the water surface	17
2.8. Leaf, coleoptile and root lengths of Lemont and red rice seedlings grown in hypoxic water. Germinated seeds were positioned 2.5 cm below the water surface	18
2.9. Maximum coleoptile lengths for Lemont and red rice seedlings grown in aerated and hypoxic water.	23
3.1. A representative transverse shoot section (A), taken between 2 and 3 mm above the seed, includes the coleoptile (c), primary leaf (pl) and second leaf (sl).	34
3.2. Representative transverse root section (A) for which the following areas were determined	36
3.3. Mean final lengths of coleoptiles and primary leaves of Lemont and red rice seedlings grown in aerated and hypoxic water. Germinated seeds were positioned at either 1.0 cm or 4.5 cm	37

3.4. Representative transverse root sections from three roots showing different arrangements of collapsed and un-collapsed cortex cells.	39
3.5. Percent of cortex occupied by gas space in root cross sections from Lemont (A and C) and red rice seedlings (B and D) grown in aerated water.	43
3.6. Percent of cortex occupied by gas space in root cross sections from Lemont (A and C) and red rice(B and D) seedlings grown in hypoxic water.	44
3.7. Area (A), number of gas space cavities (B) and percent of area occupied by gas space (C) for transverse sections of primary leaves	46
3.8. Adaxial epidermal cell length vs. maximum coleoptile length.	47
3.9. Area (A) and percent of area occupied by gas space (B) for transverse sections of coleoptiles	51

ABSTRACT

Aerenchyma - a specialized tissue with abundant, interconnected gas spaces - facilitates oxygen transport from shoots to roots in many emergent wetland plants. The importance of oxygen transport to root elongation and aerenchyma development in rice (*Oryza sativa* L.) seedlings was demonstrated in this investigation. Germinated seeds of Lemont (a commercial cultivar) and red rice (an agricultural weed) were positioned at 4.5, 2.5, or 1.0 cm below the surface of aerated or hypoxic water to control the duration of leaf submergence and the availability of oxygen. Primary root and leaf lengths were measured daily. Aerenchyma was compared among emergent and completely submerged seedlings that grew in aerated and hypoxic water. Root elongation rates were slower in hypoxic water than in aerated water. Root elongation rates increased after leaves emerged from 1.0 and 2.5 cm of overlying hypoxic water but not after leaves emerged from 4.5 cm of overlying hypoxic water. The benefits of oxygen transport to root elongation may therefore depend upon submergence depth. Measurements of transverse shoot sections taken between 2 and 3 mm above the seed revealed that 1) red rice coleoptiles grown in hypoxic water had 3 to 12 times more gas space than aerated coleoptiles and 2) the amount of gas space in primary leaves was two to five times greater in emergent seedlings that grew in hypoxic water than in aerated seedlings and completely submerged seedlings that grew in hypoxic water. Aerenchyma gas space formation occurred closer to the root tip and over a shorter distance in roots of emergent seedlings that grew in hypoxic water than in aerated roots. Roots of emergent seedlings that grew in hypoxic water also had a larger maximum amount of gas space than aerated roots. The increase in gas space between tissue ages of 10 and 45 h was similar among aerated roots and roots of emergent seedlings that grew in hypoxic water. This study is the first to report that hypoxia greatly reduced the amount of gas space and the rate of gas space formation in roots when oxygen transport was prevented by complete submergence in hypoxic water.

CHAPTER 1. INTRODUCTION TO STUDY

The soil and overlying water of flooded habitats are characterized by hypoxia and anoxia, the partial and complete depletion of oxygen. The rapid biological consumption of dissolved oxygen in the floodwater is not balanced by gas diffusion, which is 10,000 times slower in water than in air (Ponnamperuma 1972; Gambrell *et al.* 1991). Emergent “wetland” plants normally grow while rooted in flooded soils and can carry out limited periods of their lifecycle, such as when establishing a new generation or when responding to environmental changes, with little to no oxygen. For example, coleoptile elongation in germinating rice (*Oryza sativa* L.) seedlings occurs in anoxia and is stimulated by hypoxia (Ranson & Parija 1964; Ohwaki 1967; Öpik 1973; Turner *et al.* 1981; Ishizawa *et al.* 1999). Rhizomes and tubers of a small number of species can withstand periods of anoxia and some even produce shoots without oxygen (Barclay & Crawford 1982; Summers & Jackson 1994; Ishizawa *et al.* 1999). When the shoots of many rice varieties and other wetland plants become completely submerged, internodes elongate rapidly until contact with the atmosphere is restored (Jackson 1985; Voesenek *et al.* 1992). These examples of shoot elongation through oxygen-depleted soil and water are considered to facilitate the acquisition of oxygen directly from the atmosphere or via photosynthesis. For sustained development and reproduction, tissues throughout the entire plant must obtain oxygen for aerobic respiration, the main means of energy production (Vartapetian & Jackson 1997). Oxygen is transported from emergent tissues to roots, rhizomes, and submerged leaves via aerenchyma, a specialized tissue containing abundant, large gas spaces that are usually interconnected throughout a plant (for a recent review pertaining to aerenchyma see Jackson & Armstrong 1999).

Aerenchyma is either constitutive, meaning that it is always formed during development, or inducible, meaning that it only forms under specific environmental conditions. Constitutive aerenchyma occurs most often in the roots and shoots of wetland species, including rice. Hypoxia induces root aerenchyma in some non-wetland species but also increases the amount of aerenchyma in species that have constitutive aerenchyma (Smirnoff & Crawford 1983; Justin & Armstrong 1987). Comparisons of related species and varieties of single species further associate aerenchyma with habitat and flooding tolerance and reveal how aerenchyma may function in the context of other adaptations to flooding such as adventitious root formation, shoot elongation and radial oxygen loss (Datta & Banerji 1974; Visser *et al.* 1996; Watkin *et al.* 1998; Gibberd *et al.* 1999; He *et al.* 1999). These types of studies support the conclusion that oxygen transport through aerenchyma benefits plant growth in flooded, hypoxic environments. Despite this adaptive significance attributed to aerenchyma, relatively few studies have quantitatively demonstrated a correlation between oxygen transport through aerenchyma and growth rates.

Correlations between internal oxygen transport and root growth rates in rice are limited to experiments that use cylindrical electrodes to measure oxygen flux at the surface of roots (Webb & Armstrong 1983; Armstrong and Webb 1985; Waters *et al.* 1989). The internal movement of oxygen through rice plants has, however, been demonstrated in studies that did not consider growth rates (Barber *et al.* 1962; Raskin & Kende 1983a, 1985). Information specifically concerning internal oxygen transport in recently germinated rice seedlings is lacking. Although rice coleoptiles are thought to be a conduit for oxygen movement to the shoot apical meristem

when seeds germinate in saturated soil (Kordan 1974a; Takahashi 1978; Yamauchi *et al.* 1994; Yamauchi & Biswas 1997), there is no experimental evidence demonstrating oxygen transport through rice coleoptiles.

Rice has served as a model species in studies of constitutive root aerenchyma. The general pattern of cell collapse during aerenchyma formation in rice roots is consistent among investigations (Justin & Armstrong 1991; Kang *et al.* 1998; Kawai *et al.* 1998; Samarajeewa *et al.* 1999). Many studies indicate that flooding and hypoxia increase the amount of aerenchyma in rice roots (Armstrong 1971; Das and Jat 1977; Justin & Armstrong 1987; Kludze *et al.* 1994; Colmer *et al.*, 1998). However, some studies do not show this (John 1977; Jackson *et al.* 1985b). Differences among these studies in the variety, type of root, root age, method of measuring aerenchyma, and rooting medium likely confound conclusions about the effects of hypoxia on rice root aerenchyma. Additionally, in all of these studies, shoots were emergent while roots were either in soil or hydroponic solution. Even when the roots were in a hypoxic environment, oxygen could have been transported to the roots from emergent shoots via the constitutive system of aerenchyma.

Studies of rice aerenchyma have focused more on roots than other parts of the plant. We know of no studies that specifically addressed the effects of hypoxia on aerenchyma development in the leaves, stems or coleoptiles of rice. Kaufman (1959) and Matsukura (2000) have described the development of gas spaces in rice leaves. Aulakh *et al.* (2000) and Butterbach-Bahl *et al.* (2000) both studied the distribution of aerenchyma throughout rice plants to determine if the resistance to tracer gas diffusion in the root-shoot transition zone (Butterbach-Bahl *et al.*, 1997) was due to characteristics of the aerenchyma system. No connections between the aerenchyma of roots and leaf sheaths or roots and stem internodes were detected when using scanning electron microscopy (Butterbach-Bahl *et al.*, 2000). However, when bright field microscopy was used, continuity of aerenchyma was observed in the root-culm, culm-secondary tiller and culm-leaf sheath transitions (Aulakh *et al.*, 2000). Katayama (1960) showed that gas space volume in the first three leaves of rice seedlings was greater in paddy-grown plants than in upland-grown plants. When comparing the internode airspaces of rice plants that were grown in field and deep-water conditions, Datta and Banerji (1974) found differences within each of three rice varieties tested and also between the varieties.

This dissertation investigated how hypoxia and submergence affected the growth rates and anatomical development of rice seedlings. Rice seedlings between the germination and first leaf stages of development were submerged at fixed depths in either aerated or hypoxic water. In the aerated treatments, atmospheric oxygen was available to the seedlings both within and above the water. Seedlings submerged in hypoxic water could only access atmospheric concentrations of oxygen when leaves grew above the water surface. Considering previous reports of internal oxygen transport in rice and the presence of aerenchyma in the primary leaf and primary root, we presumed that leaf emergence from hypoxic water enabled oxygen diffusion between the leaf and root.

In Chapter 2, two experiments addressed the relationship between oxygen transport and root growth rates in rice seedlings. Coleoptile, primary root and leaf elongation was documented through daily measurements of length. In the first experiment, germinated seeds were initially

established at three depths to determine how depth would affect changes in root elongation rates following leaf emergence from aerated and hypoxic water. In the second experiment, seedlings were grown in either a 12 h photoperiod or continuous darkness to determine if low-intensity light would affect the change in root elongation rates following leaf emergence from aerated and hypoxic water.

In Chapter 3, anatomical development was compared among emergent and completely submerged rice seedlings grown in aerated or hypoxic water. The results provide a foundation for understanding the effects of hypoxia and submergence on primary leaf and coleoptile gas spaces. The use of primary roots in this study reduced experimental variability because each population of roots was relatively uniform in age, growth rate, and nutrient status. Many measurements of root aerenchyma have been based upon average root porosity of several roots (Das and Jat, 1977; Prade & Trolldenier 1990; Justin and Armstrong, 1991; Kludze *et al.* 1994; Colmer *et al.*, 1998), or transverse sections at only a few positions along the root (John 1977; Jackson *et al.* 1985b). Like Armstrong (1971), Kang *et al.* (1998) and Kawai *et al.* (1998), we used serial transverse sections to study root aerenchyma development. Serial transverse sections were made along the entire length of primary roots, at closer intervals where gas space development was most active. Both the distance of the transverse sections from the root tip and the age of tissues within the transverse sections were considered when describing aerenchyma formation in roots from the various treatments. Developmental differences in root anatomy associated with hypoxia should be more easily distinguished at this scale, whereas they may be obscured when only examining the mature regions of the root or when averaging porosity across whole roots.

Two strains of rice, Lemont and straw-hulled red rice (*Oryza sativa* L.; “red rice”), were used in all experiments. Lemont is an early-maturing, semidwarf, long grain cultivar that originated in Texas and has often been planted in Louisiana (LSU Ag Center Research & Extension Pub. 2270). Straw-hulled red rice and other biotypes of red rice occur naturally and are considered agricultural weeds because their relatively robust growth reduces the grain yield of commercial cultivars. The grain of red rice, exhibiting a red pericarp, is also considered a contaminant of milled “white” rice (Diarra *et al.* 1985; Kwon *et al.* 1991a, b; Kwon *et al.* 1992). Because red rice and cultivated rice are so closely related, herbicides that selectively eliminate red rice were not available until recently. In 2002, the Clearfield cultivar (BASF Corp.), developed to resist the Newpath herbicide (BASF Corp.), was grown by rice farmers for the first time (J. Oard, Louisiana State University, personal communication). Until this cultivar was available, the most effective control of red rice was achieved through water management that often involves planting pre-germinated seeds in flooded fields (Jordan & Sanders 1998). The comparisons made between Lemont and red rice seedlings in this study could provide insight into the differences and similarities between these strains as they become established in flooded fields.

CHAPTER 2. EFFECTS OF SUBMERGENCE AND HYPOXIA ON THE GROWTH OF RICE SEEDLINGS

INTRODUCTION

Plant species that normally grow in flooded habitats (“wetland” plants) are commonly exposed to hypoxia and anoxia, the partial and complete depletion of environmental oxygen. Gas diffusion, which is 10,000 times slower in water than in air, cannot compensate for the rapid biological consumption of dissolved oxygen in flooded soils and overlying water (Ponnamperuma 1972; Gambrell *et al.* 1991). Coleoptile elongation in germinating seedlings of rice (Öpik 1973; Ishizawa *et al.* 1999) and some species of *Echinochloa* (Kennedy *et al.* 1980) can occur with little to no oxygen. Rhizomes and tubers of a small number of plant species can survive extended periods of anoxia and some even generate shoots without oxygen (Barclay & Crawford 1982; Summers & Jackson 1994; Ishizawa *et al.* 1999). In many rice varieties and other wetland species, submergence of whole plants stimulates rapid shoot elongation until contact with the atmosphere is restored (Jackson 1985; Voesenek *et al.* 1992). Elongation of shoots through oxygen-depleted soil and water is believed to facilitate the acquisition of oxygen as plants establish a new generation or respond to a changing environment. Sustained development and reproduction require that tissues throughout the entire plant receive oxygen for aerobic respiration, the main means of energy production (Vartapetian & Jackson 1997). For this reason, aerenchyma, a specialized tissue containing abundant, large gas spaces, is arguably the most important anatomical adaptation for growth in flooded habitats. Gas spaces within aerenchyma are usually interconnected throughout a plant. Therefore, oxygen from the atmosphere or generated by photosynthesis enters emergent organs and is transported by diffusion and/or mass flow through aerenchymal gas spaces to rhizomes, roots and submerged leaves that have oxygen-deficient environments (for a recent review pertaining to aerenchyma see Jackson & Armstrong 1999). Oxygen diffusing out of roots is thought to further benefit the plant by oxidizing reduced phytotoxins such as hydrogen sulfide (Končalová 1990; Gambrell *et al.* 1991). Gaseous metabolic products of plant and microorganismal metabolism, such as carbon dioxide, methane, and ethylene, are also transported from the root zone to the atmosphere via aerenchyma (Higuchi 1982; Higuchi *et al.* 1984; Butterbach-Bahl 1997; Visser *et al.* 1997).

The results of previous studies support the notion that oxygen transport through aerenchyma facilitates plant growth in flooded, hypoxic environments. For example, wetland plants are commonly observed to have more root aerenchyma than non-wetland species and to increase root aerenchyma in response to flooding and hypoxia (Smirnoff & Crawford 1983; Justin & Armstrong 1987). Comparisons of related species and cultivars of single species further correlate aerenchyma with habitat and flooding tolerance and reveal how aerenchyma may function in concert with other responses to flooding such as adventitious root formation, shoot elongation and radial oxygen loss (Datta & Banerji 1974; Visser *et al.* 1996; Watkin *et al.* 1998; Gibberd *et al.* 1999; He *et al.* 1999). Despite the adaptive significance attributed to aerenchyma, relatively few studies have quantitatively demonstrated a correlation between oxygen transport through aerenchyma and increased rates of growth.

The internal movement of oxygen through rice plants in particular has been demonstrated by experiments that did not consider growth rates. Diffusion of a radioactive isotope of oxygen

from shoots to roots was greater and more easily detected in rice seedlings at the third leaf stage compared to barley seedlings that had no aerenchyma (Barber *et al.* 1962). A mass flow of air into partially submerged rice plants that were beyond the seedling stage was demonstrated by measuring changes in the volume of air surrounding emergent ends of leaves (Raskin & Kende 1983a, 1985).

Correlations between internal oxygen transport and root growth rates in rice are limited to experiments that use cylindrical electrodes to measure oxygen flux at the surface of roots. In most of these experiments, roots of rice seedlings at the two to three tiller stage were in an aqueous, anaerobic medium while shoots were enclosed within a separate chamber. Changes in oxygen flux and elongation rate at the root tip correlated with changes in oxygen concentrations surrounding the shoot. For example, when the atmosphere surrounding rice shoots was changed from air to nitrogen, oxygen concentrations at root apices decreased to zero within 15 minutes and subsequently, root extension ceased (Webb & Armstrong 1983). If the atmosphere surrounding the shoots was changed back to air within 4 h, root oxygen concentrations increased and root extension resumed. By exposing shoots and cut surfaces of excised roots to oxygen partial pressures between 1.0 kPa and air-levels, Armstrong and Webb (1985) determined that root extension declined drastically when the partial pressure of oxygen at the root apex surface dropped below a “critical oxygen pressure” that was between 0.2 and 0.8 kPa (2.4 μM to 9.8 μM O_2). The correlation between oxygen transport and root elongation was affected by plant submergence and light (Waters *et al.* 1989). When rice seedlings with three to four leaves were totally submerged, oxygen flux out of the root and root extension were greatest in the presence of light. Because the submerged plants were not in direct contact with the atmosphere, the light likely promoted photosynthetic production of internal oxygen. The influence of photosynthesis upon oxygen transport was further tested by changing variables such as carbon dioxide and oxygen concentrations in the floodwater, light intensity, and temperature. Even when light intensity was suitable for photosynthesis within the shoot, lower dissolved oxygen concentrations within the floodwater were associated with decreased oxygen flux out of the roots. Waters *et al.* (1989) reasoned that under these experimental conditions, concentration gradients promoted oxygen diffusion out of the shoot into the surrounding solution rather than internal oxygen diffusion to the roots.

Although oxygen diffusion through aerenchyma was demonstrated in older rice seedlings and plants that were beyond the seedling stage, internal oxygen transport in recently germinated seedlings has not been well studied. A potential for internal gas diffusion in young rice seedlings is suggested by the presence of aerenchyma in primary roots, primary leaves and coleoptiles (Chapter 3) which are the first three structures to emerge from a germinating embryo. In order to emerge from the embryo and grow, primary roots and leaves require more oxygen than coleoptiles (Öpik 1973). The cylindrical rice coleoptile is thought to provide an external pathway for oxygen movement to the shoot apical meristem when a seed germinates in saturated soil (Kordan 1974a; Takahashi 1978; Yamauchi *et al.* 1994; Yamauchi & Biswas 1997). However, oxygen transport through the rice coleoptile has not been demonstrated experimentally. Kordan (1974a) observed that when germinating rice seedlings were submerged in stagnant water and kept in the dark, root growth occurred only after shoots emerged above the water surface and contacted air. This and other qualitative work by Kordan (1972, 1974a, 1974b, 1976, 1977b) reinforces the fact that oxygen and light are needed for seedling

development beyond germination. However, Kordan's studies are not supported by quantitative data such as growth rates nor do they always distinguish between the coleoptile and primary leaf.

Our experiments investigated the relationship between the availability of oxygen for transport through aerenchyma and the growth of rice seedlings that were between the germination and first leaf stages of development. The availability of oxygen was manipulated by submerging germinated rice seeds at fixed depths in either aerated or hypoxic water. Atmospheric oxygen was available to seedlings both within and above aerated water. Seedlings submerged in hypoxic water could only access high concentrations of oxygen when their leaves elongated enough to emerge from the water. The aforementioned studies of oxygen transport in rice lead us to conclude that the diffusion of oxygen through aerenchyma would occur following leaf emergence from hypoxic water. When observed, changes root elongation rates at the time of leaf emergence were interpreted as a correlation between the oxygen transport through aerenchyma and root growth. Two strains of rice, Lemont and straw-hulled red rice (red rice), were used to assess how genetic differences may influence changes in root elongation rates following leaf emergence from aerated and hypoxic water. In one experiment, germinated seeds were initially established at three depths to determine how depth and the duration of complete leaf submergence would affect changes in elongation rates following leaf emergence from aerated and hypoxic water. A second experiment addressed how low-intensity light would affect changes in elongation rates after leaf emergence. Light was intended to induce developmental differences that were independent of those induced by the hypoxia and aeration.

Our two experiments were designed to further test and clarify the adaptive function of aerenchyma. They show how rates of elongation change when experimental conditions favor oxygen transport through aerenchyma. Shoot elongation is considered an important adaptive response to flooding throughout the entire life cycle of aerenchymatous wetland plants in part because when shoots contact the air, internal oxygen transport to oxygen-depleted tissues can occur. Changes in root elongation rates following shoot emergence from water into the air have not been well documented. Because these experiments were carried out using recently germinated rice seedlings, they quantitatively demonstrated that internal oxygen transport can be important for plant growth at the earliest developmental stages.

MATERIALS AND METHODS

Rice strains

Two strains of rice, Lemont and straw hull red rice (red rice), were used in these experiments. Lemont is an early-maturing, semidwarf, long grain cultivar that originated in Texas and is often planted in Louisiana (LSU Ag Center Research & Extension Pub. 2270). Lemont was chosen for this study because it consistently grew well in preliminary experiments. Straw hull and other biotypes of red rice occur naturally and are considered agricultural weeds because their robust growth reduces grain yield through competition with commercial cultivars. Additionally, because the grain of red rice has a red pericarp, it is considered to contaminate milled "white" rice (Diarra *et al.* 1985; Kwon *et al.* 1991a, b; Kwon *et al.* 1992). Red rice was chosen for this study because it has not been subjected to intentional selection for cultivation purposes (M. Cohn, Louisiana State University, personal communication) and it had the most different growth

when compared to several rice strains that were tested in preliminary experiments. Dr. Chuck Rush, Department of Plant Pathology and Crop Physiology, Louisiana State University kindly provided Lemont grain that was harvested in 1999 from the Rice Research Station (LSU Agricultural Center), Crowley, Louisiana. The Lemont grain was stored at 4°C until used in experiments that were carried out between November 2000 and August 2001. Dr. Marc Cohn, Department of Plant Pathology and Crop Physiology, Louisiana State University, generously provided straw hull red rice grain that was harvested during August 1995 from experimental plots at the Rice Research Station (LSU Agricultural Center), Crowley, Louisiana, dried to 15.1% relative moisture content, and then stored at -20°C until April 2000. To reduce dormancy, the red rice grain was transferred to room temperature for six months and then stored at 4°C until used in the experiments.

Hydroponic cultivation of seedlings

Dehulled grains (“seeds”) of rice were attached to plastic embroidery screens using acetic acid-free silicone rubber sealant (GE RTV 128 translucent). Five Lemont and five red rice seeds were alternated 1.85 cm apart across the screens and oriented so that the coleoptile emerged from the top of the embryo during germination. Screens were placed vertically over three diffusers (Sweetwater-brand, fine-pore 23.0 cm x 4.0 cm x 4.0 cm) in an 18 L plastic storage box filled with distilled, deionized water (Fig. 2.1). An inverted, 11.3 L plastic storage box was used as a loose-fitting lid. Seeds were first placed under one centimeter of water, in the dark, for 27.5 h to initiate germination. Then the seeds, with swollen embryos, and in many cases, slightly elongated coleoptiles, were positioned at 1.0, 2.5, or 4.5 cm below the water surface depending on the experiment. “Aerated” treatments were produced through continuous bubbling with air and “hypoxic” treatments were achieved through continuous bubbling with nitrogen gas. Bubbling was adjusted to be gentle and evenly distributed among the diffusers. Each day screens were rotated to different positions within the box and deionized water was added to replace evaporative loss. Experiments were carried out in a growth chamber (Environmental Growth Chambers, Chagrin Falls, OH) where water temperature in the boxes ranged between 28.0 and 29.5°C.

Experimental design

Multiple seed depths, continuous darkness Lemont and red rice seeds were attached to screens and germinated as described above. Then, the seeds were positioned at three depths, 1.0, 2.5, and 4.5 cm below the water surface. Seedlings grew in continuous darkness except for approximately 15 minutes per day when digital images were made.

Aerated and hypoxic treatments were applied simultaneously in two separate boxes. Each box contained a total of 60 seedlings that were evenly distributed among six screens. Initially, there were 10 replicate seedlings for each strain at each depth and oxygen concentration. Aerated seedlings were grown for five days following seed submergence. In the hypoxic treatment, seedlings positioned at 1.0 cm below the water surface were grown for 12 days and seedlings positioned at 2.5 and 4.5 cm below the water surface were grown for 13 days.

Single seed depth, continuous darkness or 12 h photoperiod Lemont and red rice seeds were attached to screens, germinated as described above and then positioned at 2.5 cm below the water surface. Aerated and hypoxic treatments were carried out simultaneously in two separate

boxes, first in continuous darkness and then with a 12 h photoperiod, because of time and space limitations within the growth chamber. Irradiance during the 12 h photoperiod was $30\text{--}39 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (400–700 nm) measured at 2.5 cm above the water surface.



Figure 2.1 Experimental box in which seedlings were grown. Seeds germinated while attached to plastic screens that were placed vertically over diffusers through which air or nitrogen gas was bubbled continuously. An inverted box that was used as a lid is not shown. Screens were placed on a flat bed scanner each day to collect images of the seedlings.

Each box initially contained 50 seedlings that were evenly distributed among five screens. There were initially 25 replicate seedlings for each strain at each oxygen concentration and light level. Aerated treatments were applied for five days following seed submergence while hypoxic treatments were applied for 12 days.

Dissolved oxygen concentrations in the water were measured daily using an dissolved oxygen instrument (YSI 85). In the aerated boxes, oxygen concentration averaged $7.71 \pm 0.56 \text{ mg/L}$ in continuous darkness and $6.87 \pm 0.65 \text{ mg/L}$ with a 12 h photoperiod. In the hypoxic boxes,

oxygen concentrations averaged 1.04 ± 0.25 mg/L in continuous darkness and 0.67 ± 0.08 mg/L with a 12 h photoperiod. A pH electrode was used to take daily measurements of the pH of the water in the 12 h photoperiod treatments. In both the aerated and hypoxic water, pH averaged 7.5.

Seedling growth

The coleoptile is the first structure to emerge from the germinating rice embryo but ceases to elongate shortly thereafter. The sole primary root emerges soon after the coleoptile when oxygen is present (Kordan, 1977a, 1977b). The primary leaf, which lacks a blade, elongates through the enveloping coleoptile. Depending upon genotype and environment, the internode between the coleoptilar node and seed, will sometimes elongate to form a mesocotyl (Takahashi 1978; Turner *et al.* 1982; Yamauchi *et al.* 1994; Yamauchi & Biswas 1997). The first adventitious roots appear at the coleoptilar node. When the first complete leaf, exhibiting a sheath, collar, and blade, elongates through the encircling primary leaf, the plant is considered to be in the “first leaf stage” (Dunand 1998). In our experiments, each healthy seedling produced a coleoptile, primary root, primary leaf and first complete leaf (Figs. 2.2 & 2.3). By the end of some treatments, the first complete leaf had not fully developed. Mesocotyls were not present in all treatments and, when present, were only discernable once adventitious roots began to form at the coleoptile node.

Data collection and analysis

Digital images of the seedlings were collected with a flat bed scanner that was attached to a desktop computer. Seedlings were scanned approximately every 24 h, starting 16 to 19 h after bubbling with air or nitrogen gas began (Figs. 2.2 & 2.3). The aerated treatments were terminated after 4 d of image collection because primary roots had grown as long as the screens. In hypoxic treatments, scanning continued for nine to 11 days. The slower growing primary roots grew as long as the screens in some, but not all, of the hypoxic treatments.

For each seedling, the lengths of the primary root, coleoptile, mesocotyl (if present) and leaf were measured from the scanned images using Adobe Photoshop 5.0. Primary root length was measured from the root-embryo junction to the root tip (Fig. 2.3). Coleoptile length was from the middle of the coleoptile node to the tip of the coleoptile; if no mesocotyl was present the coleoptile node was at the shoot-embryo junction. If present, mesocotyls were measured from the shoot-embryo junction to the middle of the coleoptile node. Leaf length was measured from the shoot-embryo junction to the tip of the leaf. The primary leaf and the subsequent complete leaf that grows through the primary leaf were not always distinguishable from each other in the scanned images. Therefore, a single curve assembled from daily measurements of leaf length documents the elongation of both leaves.

In both experiments, the average maximum coleoptile and mesocotyl lengths were calculated using measurements taken between 116 and 120 h for the aerated seedlings and measurements taken between 212 and 216 h for seedlings from the hypoxic treatments.

In the “single depth” experiment, the time of leaf emergence was estimated for each seedling. Leaf emergence was considered to occur when leaf length reached 2.5 cm, the depth of the water above the seed. Leaf elongation was reasonably assumed to be linear during the time that leaf

length approached and exceeded 2.5 cm. A linear regression of length vs. time, fitted to the two points that represented the leaf before and after it measured 2.5 cm, was used to estimate the time that each leaf emerged from the water.

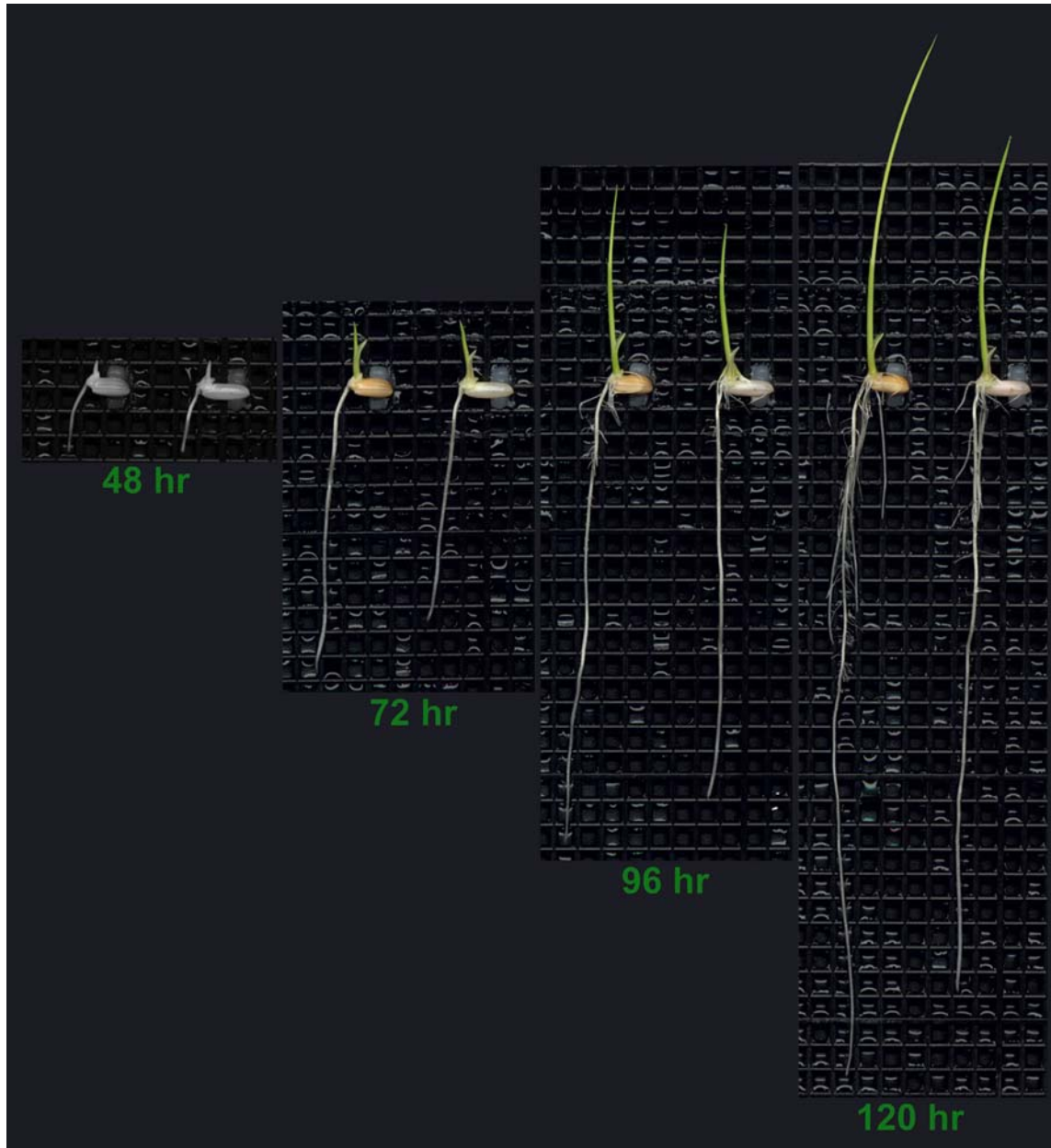


Figure 2.2 A composite figure showing scanned images of one aerated Lemont seedling (left) and one aerated red rice seedling (right) at approximately 48, 72, 96 and 120 h after imbibition. Seedlings grew in an experimental box as shown in Fig. 2.1. Images of seedlings were collected using a flat bed scanner.

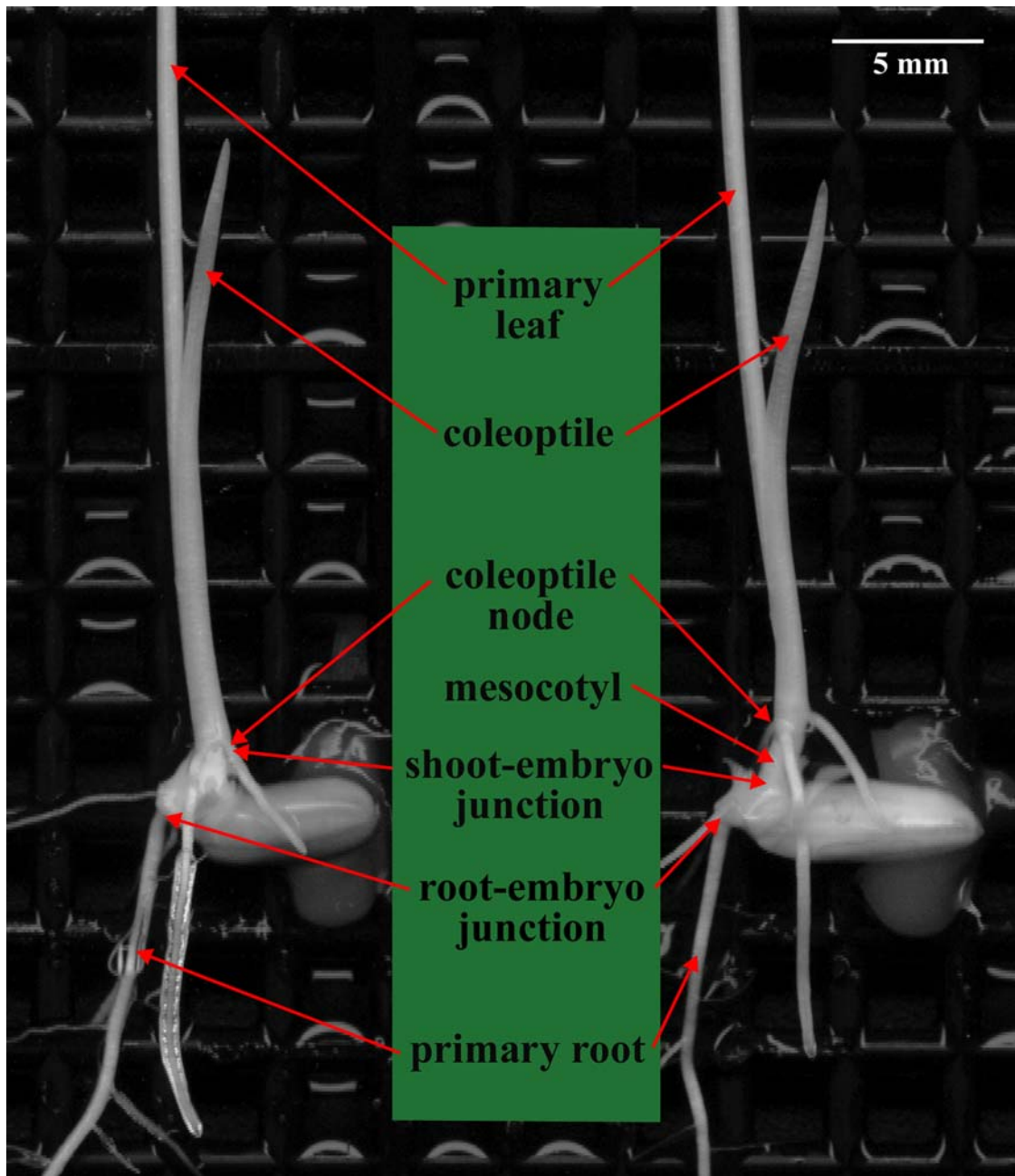


Figure 2.3 One Lemont seedling (right) and one red rice seedling (left) approximately 216 h after imbibition. Seedlings grew in continuous darkness while positioned 2.5 cm below the surface of hypoxic water. Only the Lemont seedling formed a mesocotyl. Leaf and root tips are not shown. Primary root length was measured from the root-embryo junction to the root tip. Coleoptile length was from the middle of the coleoptile node to the tip of the coleoptile; when no mesocotyl was present the coleoptile node was at the shoot-embryo junction. Mesocotyls were measured from the shoot-embryo junction to the middle of the coleoptile node. Leaf length was measured from the shoot-embryo junction to the tip of the leaf.

RESULTS

Leaves and primary roots

Multiple seed depths, continuous darkness

Aerated treatment For both aerated Lemont and red rice, the change in leaf and root lengths during approximately 120 h of growth was similar among seedlings positioned at 1.0, 2.5 and 4.5 cm below the water surface (Fig. 2.4). The leaves of red rice seedlings from all three depths had emerged from the water by 120 h. However, the leaves of the Lemont seedlings that were started at 4.5 cm below the water surface had not emerged from the water by 120 h. The slopes representing the change in root length over time appeared linear and did not change when leaves emerged from the water.

Hypoxic treatment All roots growing in hypoxic water had slower elongation over time compared to roots growing in aerated water that reached maximum lengths by approximately 120 h. For both Lemont and red rice, the pattern of change in root and leaf lengths over time differed among seedlings that were started at three different depths in hypoxic water (Figs. 2.5 & 2.6).

Seedlings started at 1.0 and 2.5 cm below the surface of hypoxic water, had a greater change in root length over time after leaves emerged from the water compared to before leaves emerged. For example, the leaves of Lemont seedlings (Fig. 2.5A) that were started at 1.0 cm emerged from the water between 72 and 96 h and the roots of these seedlings (Fig. 2.5B) began elongating more quickly during this interval. Roots of Lemont seedlings started at 2.5 and 4.5 cm displayed a similar change in length over time while the leaves of these seedlings were completely submerged. However, at approximately 192 h, the leaves of Lemont seedlings that were started at 2.5 cm emerged from the water and the roots of these seedlings began elongating faster than they had prior to leaf emergence. The leaves of Lemont seedlings that were started at 4.5 cm emerged into the air between 264 and 288 h but the roots of these seedlings did not begin to elongate more quickly at this time.

As with Lemont, in red rice seedlings started 1.0 and 2.5 cm below the surface of hypoxic water, roots elongated more rapidly after leaf emergence than they had prior to leaf emergence (Fig. 2.6). And, roots of red rice seedlings started at 2.5 and 4.5 cm showed a similar change in length over time while the leaves of these seedlings remained completely submerged. The leaves of red rice seedlings that were started at 4.5 cm barely emerged from the water by the end of the experiment and the roots of these seedlings did not exhibit a change in elongation at this time.

Single seed depth, continuous darkness or 12 h photoperiod

Aerated treatment In aerated seedlings from the single depth experiment, the changes in leaf and root lengths over approximately 120 h were similar to those of aerated seedlings from the multiple depth experiment. Lengths of leaves, after 72 h, and roots, after 48 h, were longer in red rice than in Lemont both in continuous darkness (Fig. 2.7A & B) and with a 12 h photoperiod (Fig. 2.7C & D). The leaves of aerated red rice seedlings emerged from the water approximately 10 h earlier than the leaves of aerated Lemont seedlings (Table 2.1). The presence or absence of light did not affect the time that leaves emerged from the water in either

Lemont or red rice seedlings (Table 2.1). The slopes representing change in root length over time appeared linear and did not change when leaves emerged from the water.

Hypoxic treatment With both continuous darkness (Fig. 2.8A & B) and a 12 h photoperiod (Fig. 2.8C & D), roots growing in hypoxic water displayed a slower change in length over time compared to aerated roots that grew to maximum lengths by approximately 120 h. The increase in root length over time occurred more quickly after leaves emerged from the hypoxic water compared to before leaves emerged (Fig. 2.8B & D). Root elongation after leaf emergence from hypoxic water was not as fast as the elongation of aerated roots during the first 120 h of the experiment (Fig. 2.7B & D). Differences in the changes in leaf and root length over time between Lemont and red rice became greater after leaves grew out of the water (Fig. 2.8). For both Lemont and red rice, leaf and root lengths at the end of the hypoxic treatment were shorter when seedlings grew with a 12 h photoperiod than when seedlings grew with continuous darkness. At the end of the experiment, seedlings from the hypoxic treatments had longer leaves and shorter roots relative to seedlings from the aerated treatments.

Table 2.1 Mean leaf emergence times (± 1 S.D.) for Lemont and red rice seedlings. Aerated and hypoxic treatments were applied under continuous darkness or a 12 h photoperiod. Leaf emergence was considered to occur when leaf length reached 2.5 cm, the depth of the water above the seed. Times were estimated as described in the Materials and Methods.

		mean emergence time (h) ± 1 S.D.	
	treatment	Lemont	red rice
aerated	continuous darkness	101 \pm 5	90 \pm 3
	12 h photoperiod	101 \pm 8	91 \pm 5
hypoxia	continuous darkness	167 \pm 19	182 \pm 13
	12 h photoperiod	192 \pm 9	187 \pm 18

The changes in leaf lengths over time were more variable among seedlings exposed to hypoxia than among aerated seedlings. Therefore, for the hypoxic treatment, the mean emergence times that were estimated using individual seedlings were slightly different than the emergence times that are indicated by Figure 2.8, the change in mean leaf length over time. When seedlings were exposed to hypoxia and continuous darkness, the mean time of leaf emergence was 15 h earlier in Lemont seedlings than in red rice seedlings (Table 2.1). The estimated mean emergence times were similar between Lemont and red rice when seedlings were exposed to hypoxia and a 12 h photoperiod.

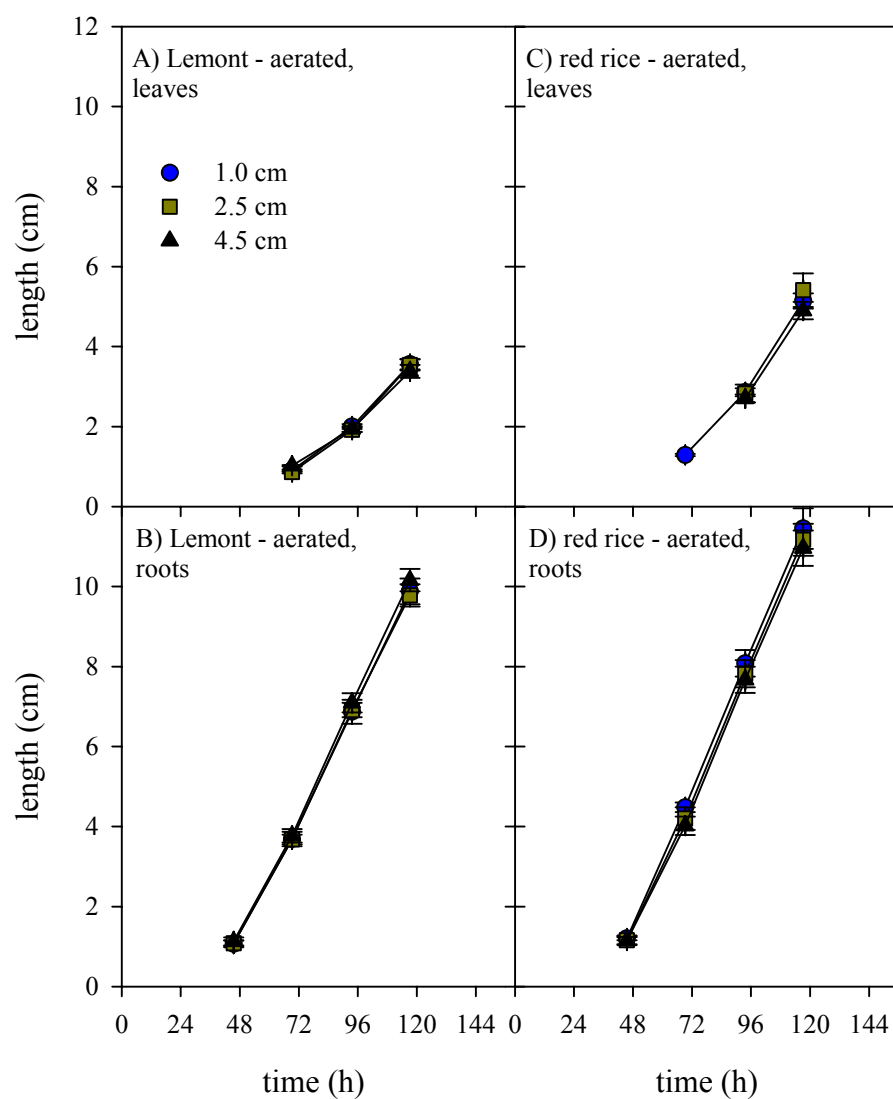


Figure 2.4 Leaf and primary root lengths of Lemont (A, B) and red rice seedlings (C, D) grown in aerated water. Germinated seeds were positioned at three depths (1.0, 2.5 and 4.5 cm) below the water surface. Values are mean lengths (± 1 S.E.) of the leaves or roots of 5 to 10 seedlings.

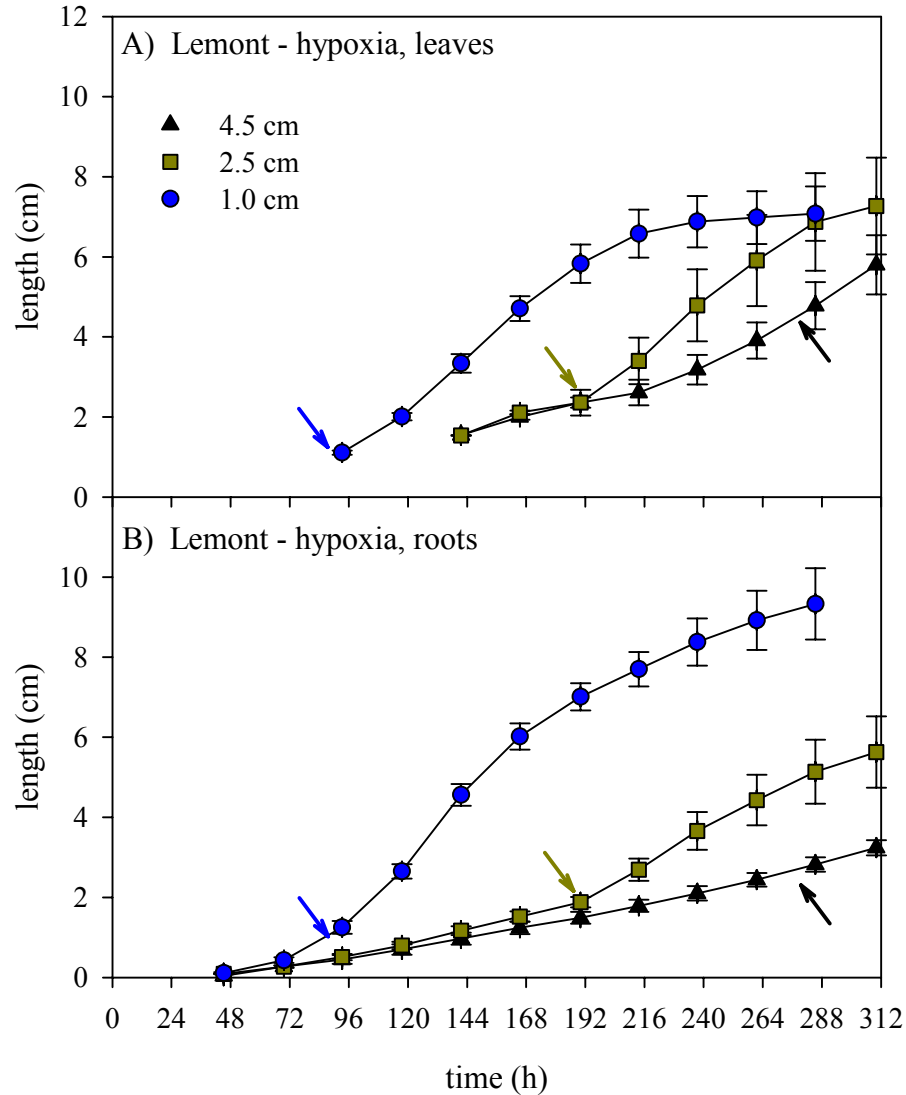


Figure 2.5 Leaf (A) and primary root (B) lengths of Lemont seedlings grown in hypoxic water. Germinated seeds were positioned at three depths (1.0, 2.5 and 4.5 cm) below the water surface. Arrows indicate the time that average leaf length equaled submergence depth. Values are mean lengths (± 1 S.E.) of the leaves or roots of 5 to 10 seedlings.

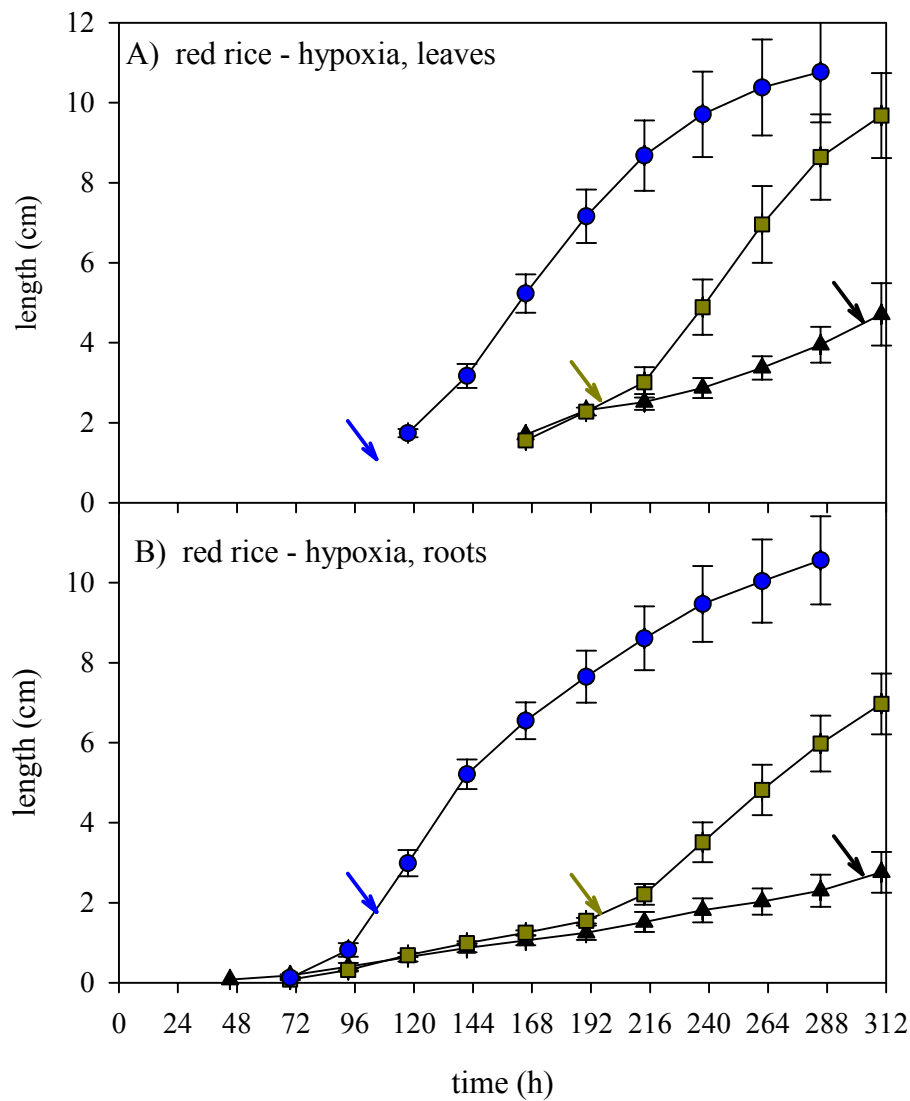


Figure 2.6 Leaf (A) and primary root (B) lengths of red rice seedlings grown in hypoxic water. Germinated seeds were positioned at three depths (1.0, 2.5 and 4.5 cm) below the water surface. Arrows indicate the time that average leaf length equaled submergence depth. Values are mean lengths (± 1 S.E.) of the leaves or roots of 5 to 10 seedlings.

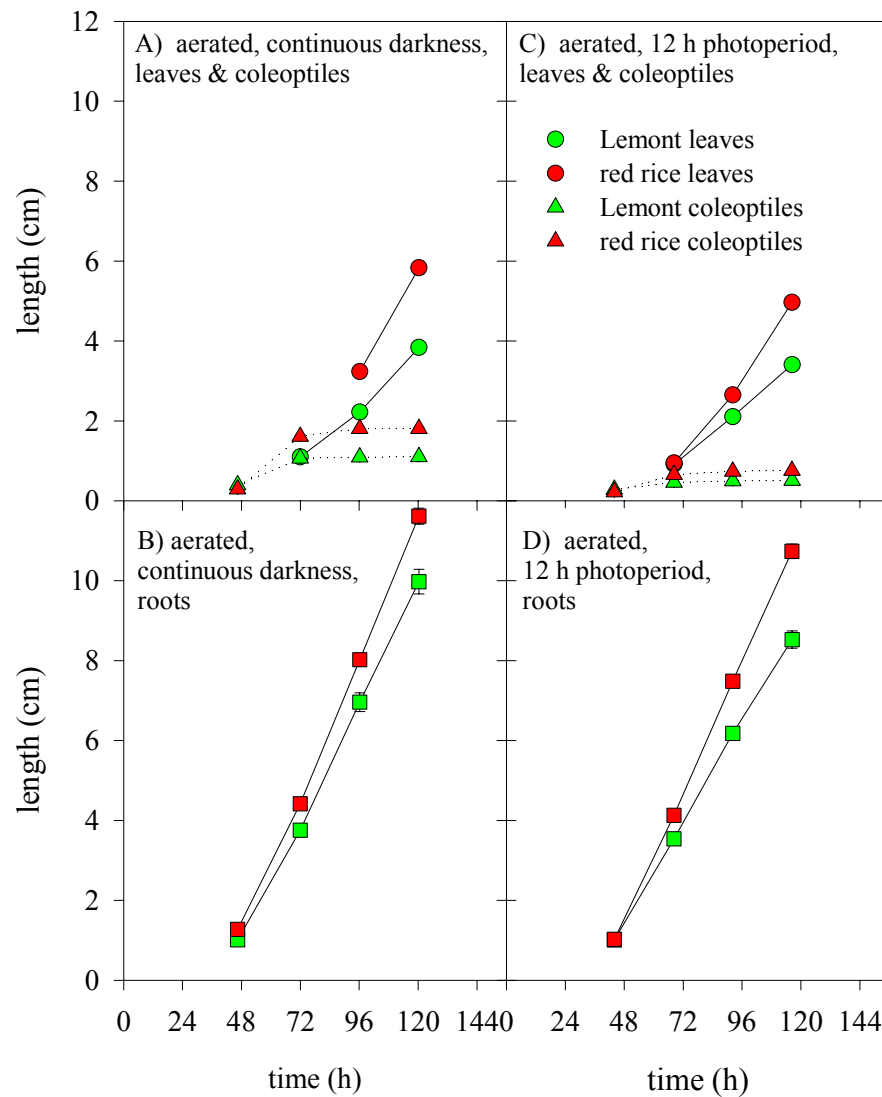
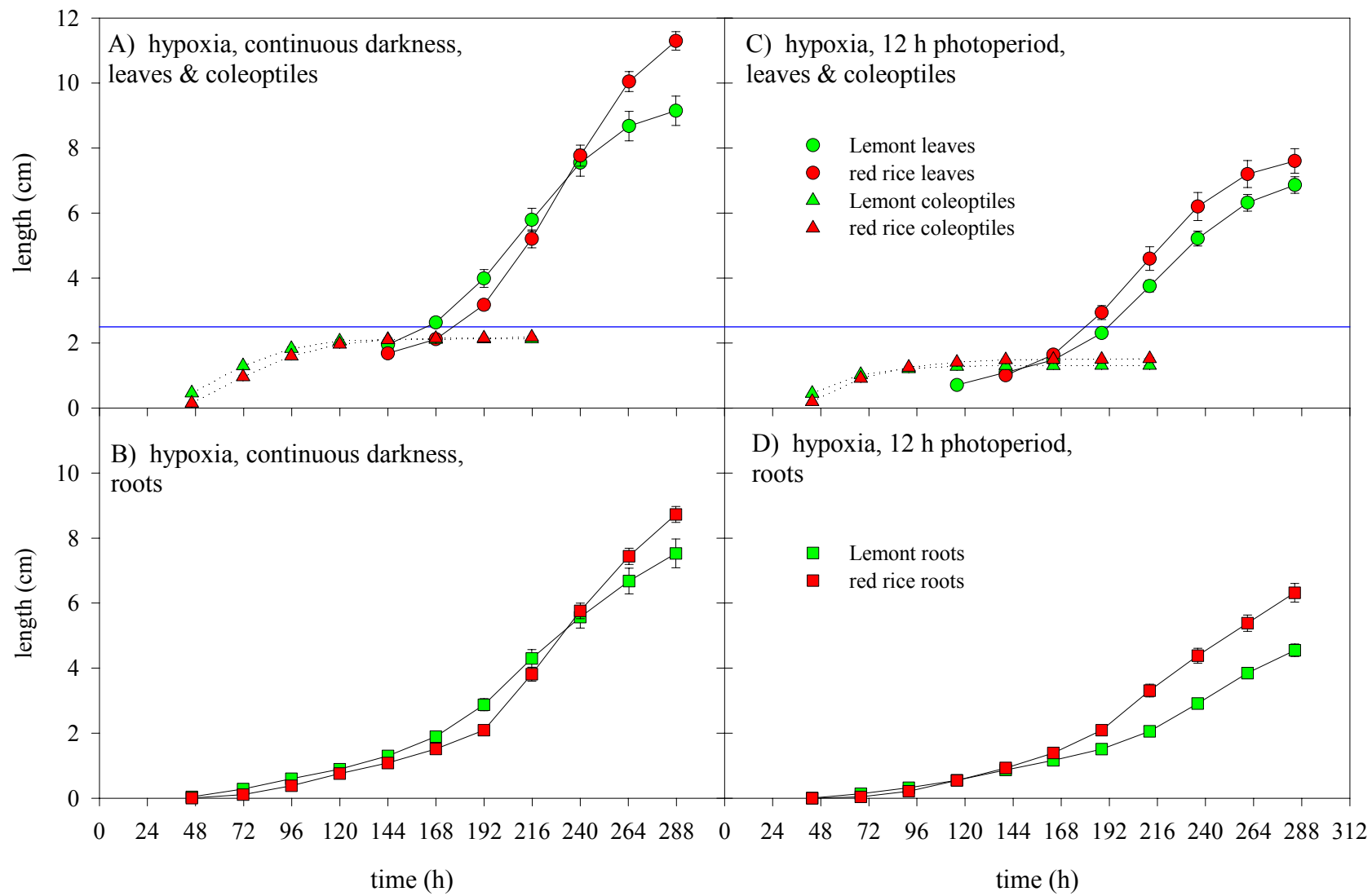


Figure 2.7 Leaf, coleoptile and root lengths of Lemont and red rice seedlings grown in aerated water. Germinated seeds were positioned 2.5 cm below the water surface and exposed to either continuous darkness (A, B) or a 12 h photoperiod (C, D). Values are mean lengths (± 1 S.E.) of 20 to 25 seedlings.

Figure 2.8 Leaf, coleoptile and root lengths of Lemont and red rice seedlings grown in hypoxic water. Germinated seeds were positioned 2.5 cm below the water surface and exposed to either continuous darkness (A, B) or a 12 h photoperiod (C, D). Leaves contacted the air once their length surpassed the 2.5 cm of overlying water (reference lines in A and C). Values represent mean lengths (± 1 S.E.) of 18 to 24 seedlings.



C

Coleoptiles and Mesocotyls

Multiple seed depths, continuous darkness

Within each treatment, coleoptile length increased as seed depth increased (Fig. 2.9A). For both Lemont and red rice, seedlings exposed to hypoxia had longer coleoptiles than aerated seedlings (Fig. 2.9A).

Single seed depth, continuous darkness or 12 h photoperiod

In both Lemont and red rice, seedlings exposed to hypoxia had longer coleoptiles than aerated seedlings in both the presence and absence of light (Fig. 2.9B). When there was continuous darkness, the difference in coleoptile length between hypoxic and aerated seedlings was greater for Lemont than for red rice (Fig. 2.9B). Within each treatment, coleoptiles exposed to 12 h of light per day were shorter than those exposed to continuous darkness (Fig. 2.9B). When comparing seedlings that received the same light and oxygen level treatments mean maximum coleoptile length was always greater in red rice than in Lemont (Fig. 2.9).

Mesocotyls did not form in seedlings that were exposed to a 12 h photoperiod. The effect of oxygen level on mesocotyl formation differed between Lemont and red rice seedlings that were exposed to continuous darkness; Lemont seedlings formed more mesocotyls in the hypoxic treatment while red rice seedlings formed more mesocotyls in the aerated treatment (Table 2.2). The greatest mean mesocotyl length occurred in hypoxically-treated Lemont seedlings and it was between 2 and 3.5 times larger than the mean mesocotyl lengths of the other treatments (Table 2.2). Similar effects of oxygen level upon mesocotyl formation and length were observed in the multiple depth experiment (data not shown). When mesocotyls formed in hypoxically-treated Lemont and aerated red rice seedlings, the mesocotyls of seedlings started at 2.5 and 4.5 cm were longer than the mesocotyls of seedlings started at 1.0 cm (data not shown).

Table 2.2 Mesocotyl formation and length in Lemont and red rice seedlings grown in aerated and hypoxic water. Germinated seeds were positioned 2.5 cm below the water surface and exposed to continuous darkness. Mesocotyls did not form when seedlings were exposed to a 12 h photoperiod. Only seedlings with mesocotyls were used when calculating mean lengths.

treatment	% seedlings that formed mesocotyls	mean mesocotyl length (cm) \pm 1 S.E.
Lemont - aerated	12.5	0.057 \pm 0.003
red rice - aerated	69.6	0.095 \pm 0.010
Lemont - hypoxia	100.0	0.211 \pm 0.011
red rice - hypoxia	41.7	0.083 \pm 0.011

When present, mesocotyls were short relative to coleoptiles. For example, in the single depth experiment, mean mesocotyl length ranged from 0.06 to 0.19 cm whereas the mean coleoptile length ranged from 1.10 to 2.15 cm in the treatments in which mesocotyls formed.

DISCUSSION

Primary roots and leaves

During the time that Lemont and red rice seedlings were submerged in hypoxic water they had slower primary root and leaf elongation than seedlings that were submerged in aerated water. These results are consistent with previous studies that show that lengths of roots and leaves or rates of root and leaf elongation in rice seedlings decline with decreasing oxygen concentration (Ranson & Parija 1955; Turner *et al.* 1981; Armstrong & Webb 1985; Yamauchi & Biswas 1997). Our study of the effects of oxygen concentration and submergence on rice seedlings is unique in its evaluation of how the rate of root elongation changes when leaves emerge into the air.

Submerged rice seedlings that were between the germination and first leaf stages of development could access atmospheric concentrations of oxygen either within and above the surface of aerated water or only above the surface of hypoxic water. Once leaf emergence from the water established a connection between the seedling and the atmosphere, an oxygen concentration gradient within the tissues of the seedling would have driven internal oxygen diffusion towards submerged tissues. The change in length over time of primary roots and of leaves was similar among aerated rice seedlings positioned at three different depths (Fig. 2.4). Root elongation rates did not change when leaves emerged from aerated water (Fig. 2.4 & 2.7). Dissolved oxygen obtained from the aerated water was presumably sufficient to support the maximum rates of root elongation that the aerated seedlings displayed. The average root elongation rate was calculated as 1.2 mm h⁻¹ in aerated Lemont seedlings and 1.4 mm h⁻¹ in aerated red rice seedlings. Iijima *et al.* (1998) observed a similar average primary root elongation rate of 1.3 mm h⁻¹ in rice seedlings that were between 6 and 7 days old. When seedlings were started at 1.0 cm and 2.5 cm below the surface of hypoxic water, elongation rates of primary roots increased within the same interval of time as leaf emergence from the hypoxic water (Fig. 2.5). Oxygen diffusing from the emergent portion of the leaf to the root meristem presumably stimulated faster root elongation. These results suggest that diffusion-driven, internal oxygen transport can enhance seedling growth provided that a portion of the leaf is contacting the atmosphere and that remaining portions of the seedling are oxygen-depleted. However, data from seedlings started 4.5 cm below the surface of hypoxic water suggest that a deeper submergence depth or a longer duration of complete submergence may reduce the likelihood that oxygen transport will benefit seedling growth once leaves emerge from the water.

When seedlings were started 4.5 cm below the surface of hypoxic water an increase in the rate of root elongation did not occur when leaves emerged from the water (Fig. 2.5). Leaves of Lemont seedlings from this treatment emerged over 12 h prior to the end of the experiment while leaves of red rice seedlings from this treatment emerged within the last 12 h of the experiment. While a longer experiment could determine if root elongation rates would eventually increase more than 12 h after leaf emergence, several factors may explain why root elongation rates did not increase

soon after leaf emergence, particularly in Lemont. In a recent review, Jackson and Armstrong (1999) summarize that physical resistance and oxygen demands along the root influence the effectiveness of internal oxygen transport. Physical resistance is a function of path length, pore (gas space) size, porosity (reflects total gas space volume) and path tortuosity while oxygen demands arise in root tissues and the rhizosphere. The distance between the water surface and the root tip, the “path length”, was greatest in seedlings started at 4.5 cm. In a companion study (Chapter 3), we found that aerenchyma development was inhibited in seedlings that grew while completely submerged in hypoxic water for 236 h. Therefore, seedlings that were started at 4.5 cm below the surface of hypoxic water in this experiment likely had a large volume of oxygen consuming cells, a small average pore size and lower porosity. After leaves emerged from 4.5 cm of overlying hypoxic water, a greater distance for diffusion, a small volume of gas space, oxygen consumption by submerged tissues, and diffusion of oxygen out of the seedling may have collectively inhibited internal oxygen diffusion to the root tip and hence prevented the increased root growth that was observed after leaves emerged from 1.0 cm and 2.5 cm of overlying hypoxic water. Root tips of seedlings started at 4.5 cm below the water surface remained turgid but cells within the meristematic region may have been stressed by prolonged hypoxia, periods of anoxia, or aeration following anoxia. Even though rice can grow in oxygen depleted environments, cells within rice roots are susceptible to damage from anoxia especially when internal oxygen transport is inhibited. In intact rice plants with shoots contacting the air, root cell ultrastructure was disrupted within 48 h after oxygen was displaced from the root zone (Vartapetyan *et al.* 1969). In detached rice roots where oxygen transport was not possible, mitochondrial structure in the cortex cells was disrupted after just 12 h of anoxia (Vartapetian *et al.* 1977). The oxygen radicals produced upon aeration of anoxic tissues can cause cellular damage and lead to cell death (Pfister-Sieber & Brändle 1994). Rice seedlings have a superoxide radical detoxification system but during adaptation to air following 6 days of submergence, the activities of six enzymes in this system took 24 h to reach a maximum (Ushimaru *et al.* 1992). Therefore, when seedlings were started 4.5 cm below the water surface, root elongation following leaf emergence may also have been delayed while meristematic cells recovered from structural damage and adjusted to aeration.

In addition to internal oxygen diffusion through aerenchyma to the root tip, other factors may have lead to faster root elongation rates following leaf emergence from hypoxic water. Although starch hydrolysis and sucrose synthesis occur in anoxic rice seedlings (Guglielminetti *et al.* 1995; Pereta *et al.* 1998), an increase in oxygen concentration following leaf emergence may have increased the rates of these processes or rates of sucrose transport within the endosperm and seedling. This could have contributed to an increase in root elongation rates by increasing substrate for aerobic and/or anaerobic metabolism within meristematic tissues. External transport of gasses after leaf emergence from hypoxic water may also have supplied submerged tissues with atmospheric oxygen in these experiments. A layer of air on the surface of submerged portions of emergent leaves has been shown to facilitate gaseous diffusion and the mass flow of air into partially submerged rice plants (Raskin & Kende 1983a, 1985). The plants that Raskin and Kende (1983a, 1985) used were fully developed with leaves, stems and a root system. We are not aware of any studies that have addressed whether or not partially submerged, recently germinated seedlings form an external layer of air through which the movement of oxygen can occur.

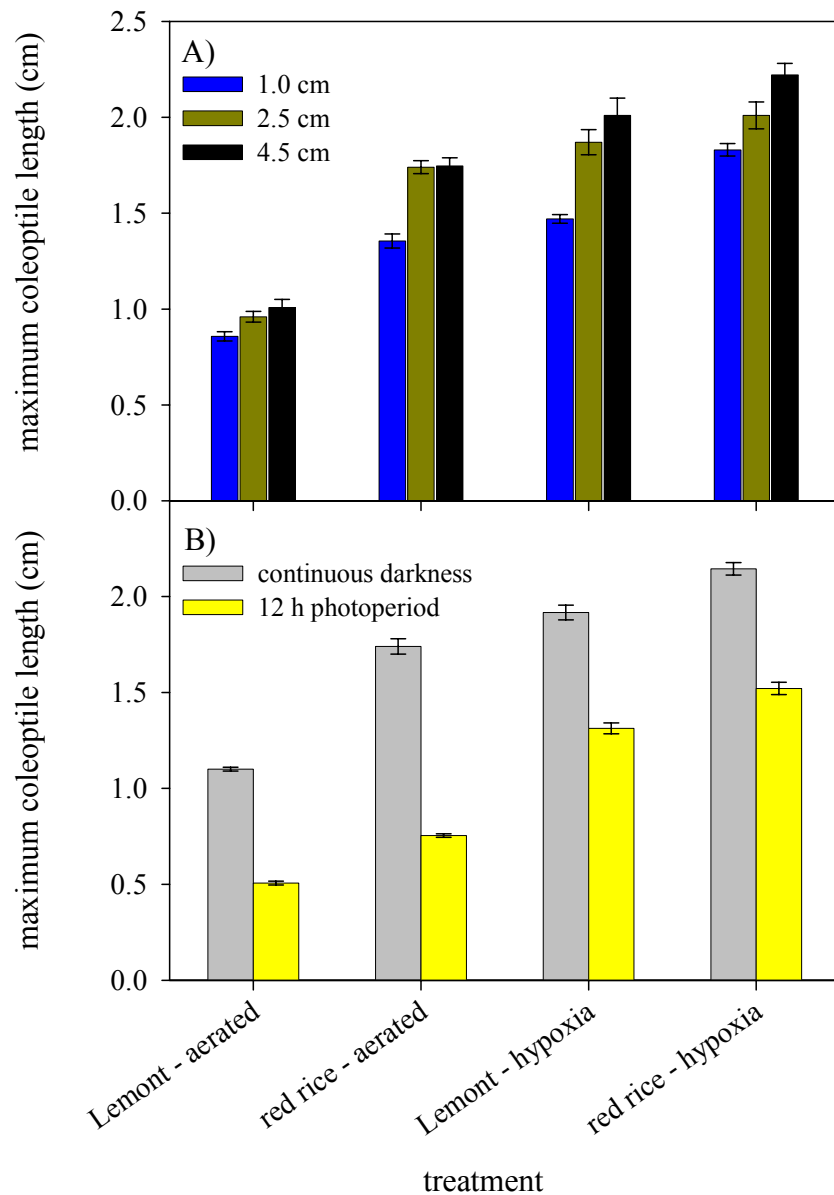


Figure 2.9 Maximum coleoptile lengths for Lemont and red rice seedlings grown in aerated and hypoxic water. Germinated seeds were positioned at three different depths (1.0, 2.5, and 4.5 cm) in continuous darkness (A) or at 2.5 cm below the water surface with either continuous darkness or a 12 h photoperiod. Bar height represents mean length (± 1 S.E.) of 5 to 10 seedlings in A and 20 to 25 seedlings in B.

The 12 h photoperiod was not expected to effect growth by augmenting photosynthetic oxygen and carbohydrate production in this study because the low light intensity would not support high rates of photosynthesis and because rice seedlings can use endosperm resources even in the absence of oxygen (Guglielminetti *et al.* 1995; Pereta *et al.* 1998). The most noticeable effect of light was to decrease coleoptile and mesocotyl lengths. We anticipated greater differences between seedlings grown with and without light because etiolated seedlings of other species typically have pronounced internode elongation. The light that seedlings were exposed to during scanning probably interfered with photomorphogenic effects of continuous darkness. In this study, final leaf and root elongation lengths were similar between aerated seedlings grown in continuous darkness and with a 12 h photoperiod during the first 120 h following imbibition (Fig. 2.7). Light effects on root and leaf elongation may or may not have become apparent if the aerated seedlings grew for a longer period of time. More typical of etiolated seedlings, leaf and root lengths at the end of the hypoxic treatment were shorter with a 12 h photoperiod than with continuous darkness (Fig. 2.8). The increased oxygen availability following leaf emergence from hypoxic water may have facilitated longer-term effects of light on root and leaf elongation. The results of Kordan (1972, 1974a, 1975, 1976) suggest that hypoxia interferes with normal seedling response to light. He observed that there was little difference between light- and dark-grown seedlings that were submerged under water that had reduced gas exchange with the atmosphere; coleoptiles formed but there was no root growth, little to no leaf growth and no greening of shoot tissues. Subsequently, when both the light- and dark-grown seedlings were exposed to light and the barrier to gas exchange was removed, roots grew and shoots turned green and grew vertically.

Lemont and red rice seedlings at the earliest developmental stages demonstrated the capacity for internal oxygen transport. The relative importance of internal oxygen transport to Lemont and red rice seedlings must be considered in the context of each rice strain's response to factors like submergence, oxygen concentration and light. In aerated water, the acquisition and transport of oxygen, facilitated by leaf contact with the atmosphere, did not appear to be important for seedling growth in either strain (Figs. 2.4 & 2.5). Additionally, the presence or absence of light did not affect the average time of leaf emergence from aerated water in Lemont or red rice (Fig. 2.7, Table 2.1). Reminiscent of the robust growth that red rice biotypes exhibit in the field, red rice emerged from aerated water sooner than Lemont (Table 2.1) and the root and leaf lengths of red rice became increasingly larger than those of Lemont during 120 h of growth in aerated water. Root elongation was inhibited when seedlings were completely submerged in hypoxic water and a deeper submergence increased the time it took for leaves to emerge from the water (Figs. 2.5 & 2.6). The slow change in root lengths between 48 h and 168 h was similar between hypoxically-treated Lemont and red rice seedlings positioned at a depth of 2.5 cm, both in the presence and absence of a 12 h photoperiod (Fig. 2.8). Leaf emergence from 1.0 and 2.5 cm of overlying hypoxic water was associated with faster root elongation in both Lemont and red rice (Figs. 2.5, 2.5 & 2.7). With continuous darkness, Lemont seedlings in the single depth experiment had a "head start" because the average leaf emergence time was earlier in Lemont than in red rice (Table 2.2). This may explain why the average leaf and root lengths of red rice only exceeded those of Lemont after 240 h (Fig. 2.8A & B). A 12 h photoperiod delayed leaf emergence in Lemont (Table 2.2). Consequently, red rice had an earlier average emergence time than Lemont and between the time of leaf emergence and the end of the experiment, red rice

continued to have larger average leaf and root lengths than Lemont (Fig. 2.8C & D). These data suggest that the relative time of leaf emergence, affected by submergence depth and the availability of light, may be important to the competitive interactions among seedlings that are becoming established in the field. Following leaf emergence from 4.5 cm of overlying hypoxic water, seedlings in the multiple depth experiment did not exhibit a faster increase in root lengths (Figs 3 & 4). Water that is too deep and depleted of oxygen may prevent the acquisition and transport of oxygen and consequently delay or even prohibit the establishment of both Lemont and red rice seedlings in the field.

Coleoptiles and mesocotyls

Red rice coleoptiles were always longer than Lemont coleoptiles among seedlings that received the same treatment, but coleoptiles of both varieties were shorter when grown with a 12 h photoperiod than when grown in continuous darkness (Fig. 2.9). Similarly, Raskin & Kende (1983b) showed that seedlings exposed to continuous darkness had longer coleoptiles than seedlings exposed to continuous light. The inhibition of coleoptile elongation by light did not preclude the stimulation of coleoptile elongation by hypoxia. Final coleoptile length in hypoxic treatments was longer than in aerated treatments by 73% in Lemont and 24% in red rice when seedlings were grown under continuous darkness and by 159% in Lemont and 102% in red rice when seedlings were grown with a 12 h photoperiod.

Previous research has repeatedly documented that, although rice coleoptiles can elongate under anoxic conditions, they usually elongate more under hypoxic conditions. For the two varieties tested by Ranson and Parija (1955), final coleoptile lengths at 30°C were greatest at 3.5% oxygen in air and lengths at 0.5% oxygen were even shorter than those at 21% oxygen. The elongation of excised sections of coleoptiles was stimulated at all oxygen concentrations below atmospheric levels (21% oxygen in air and 5.48 ml oxygen/L in water) and maximum elongation occurred at 0.65 ml oxygen/L in water when coleoptiles were submerged and 2.5% oxygen in gas when coleoptiles were floated on water (Ohwaki 1967). Ishizawa and Esashi (1984) found that coleoptile elongation was stimulated when the incubation medium was saturated with gas containing 0.02 to 0.13 m³m⁻³ (2 to 13 %) oxygen and maximum elongation occurred when the medium was saturated with gas containing between 0.04 and 0.06 m³m⁻³ (4 to 6 %) oxygen. Considering these previous experiments, the oxygen concentrations in our hypoxic treatments (32.5 ± 7.8 µM in continuous darkness and 20.9 ± 2.5 µM with a 12 h photoperiod) would be expected to elicit near-maximum amounts of elongation in Lemont and red rice coleoptiles.

The difference in coleoptile response to hypoxia between Lemont and red rice is also consistent with the results of other studies. All ten rice cultivars tested by Turner *et al.* (1981) had longer coleoptiles when seedlings were submerged in water saturated with air containing 1.0 % oxygen (12.2 µM oxygen in solution) than when seedlings were submerged in water saturated with air containing in 10.5% oxygen treatment (128.4µM oxygen in solution). Among the 10 cultivars, the percent increase in coleoptile length ranged from 47% to 135%. Among the 17 cultivars tested by Atwell *et al.* (1982), coleoptiles were between 40 % and 230% longer when seedlings were submerged in stagnant water (oxygen measured approximately 0.125 mmol L⁻¹ after 48 h) compared to when seedlings were grown in water-saturated air.

In this study, mesocotyls only formed when seedlings grew in continuous darkness (Table 2.2). Raskin and Kende (1983b) also demonstrated that mesocotyl formation was inhibited by continuous light when seedlings were submerged in aerated water. Interestingly, Lemont seedlings formed more mesocotyls in the hypoxic treatments while red rice seedlings formed more mesocotyls in the aerated treatments. Varietal differences in mesocotyl elongation are known to occur. Mesocotyl elongation tends to be more pronounced in *indica* cultivars than in *japonica* cultivars (Suge 1971, 1972; Takahashi 1978). Turner *et al.* (1982) showed that two standard height cultivars had a greater capacity for mesocotyl elongation than three semidwarf cultivars. When imbibed rice seeds were grown in Erlenmeyer flasks that contained oxygen concentrations ranging from 0.0 % to 21.0 % in air, one of four rice varieties formed no mesocotyls at any oxygen concentration and, like red rice in our experiments, the other three varieties formed the longest mesocotyls at the highest oxygen concentration (Yamauchi & Biswas 1997).

In the multiple depth experiment, maximum coleoptile length increased with the depth of seedling submergence in both aerated and hypoxic water (Fig. 2.9). The data suggest that mesocotyl formation also varied among seedlings positioned at 1.0, 2.5 and 4.5 cm below the surface of water however, the sample size was not large enough to apply descriptive statistics to mesocotyl formation, which did not occur in all seedlings. Raskin and Kende (1983b) likewise found that coleoptile and mesocotyl length increased with depth of submergence in aerated water. Most other experiments used soil as a substrate when evaluating the effects of planting depth on coleoptile and mesocotyl length. Coleoptile length increased with planting depth in water-saturated Vermiculite (Raskin & Kende 1983b). Yamauchi and Biswas (1997) found that coleoptile and mesocotyl length increased with sowing depth in drained but not in flooded soils. When seeds of five rice cultivars and straw hull red rice were planted in saturated soil at depths ranging from one to 10 cm, the sum of coleoptile and mesocotyl length was equal to seed depth except in the case of two semidwarf varieties which did not elongate through 10 cm of overlying soil (Turner *et al.* 1982).

Ethylene and carbon dioxide as well as abscisic acid and gibberellic acid stimulate elongation of coleoptiles (Ku *et al.* 1970; Suge 1971; Katsura and Suge 1979; Ishizawa and Esashi 1983, 1984; Yamauchi *et al.* 1994) and mesocotyls (Suge 1971, 1972; Takahashi 1972, 1973; Yamauchi *et al.* 1994). In combination, these compounds have an additive effect on elongation. Coleoptiles and mesocotyls generally elongate more when experimental conditions promote the accumulation of ethylene and carbon dioxide and the consumption of oxygen around seedlings (i.e. sealed, gas-containing containers and submergence in stagnant water or soil) than in conditions that flush the atmosphere surrounding seedlings (i.e. containers with a constant flow of gas and submergence in bubbled solutions) (Ku *et al.* 1970; Zarra & Masuda 1979; Atwell *et al.* 1982; Turner *et al.* 1982; Raskin & Kende 1983b; Ishizawa & Esashi 1984; Setter & Ella 1994). In our experiment, the continuous bubbling of either air or nitrogen gas likely reduced overall variation in the water column and displaced ethylene and carbon dioxide released into the water by the seedlings. This may explain why the maximum coleoptile and mesocotyl lengths achieved in our experiments were small relative to many other studies. For example, at a sowing depth of 6 cm in soil, red rice coleoptile length averaged 3.81 cm and mesocotyl length averaged 2.13 cm (Turner *et al.* 1982). Among all of our treatments, the longest average coleoptile and mesocotyl lengths for red rice were 2.22 cm and 0.11 cm, respectively. Differences in coleoptile and mesocotyl lengths

among the different sowing depths are likely due in part to depth-dependent variation in ethylene, carbon dioxide, and oxygen concentrations in the soil. The variation we observed in the coleoptiles and mesocotyls of seedlings started at the three different depths may be attributed to environmental gradients that developed in unmixed layers of solution surrounding the seedlings.

The coleoptile, sometimes accompanied by a mesocotyl, is the first structure to emerge from the embryo during rice germination. The coleoptile ceases elongation and eventually senesces as the seedling continues to grow. Therefore, as more leaves and roots develop and mature, the coleoptile and mesocotyl become smaller components of overall plant structure. The fact that these determinant organs respond to many environmental stimuli suggests that they have short-lived but important functions. Yamauchi and Winn (1996) showed that there was a significant correlation between coleoptile length and seedling establishment in the field where cultivars with longer coleoptiles had better establishment. Turner *et al.* (1982) demonstrated that a seedling's ability to emerge from soil depends on both mesocotyl and coleoptile elongation. The function most often attributed to the elongating rice coleoptile is the acquisition of oxygen when seeds germinate in saturated, oxygen-depleted soil (Kordan 1974a; Takahashi 1978; Yamauchi *et al.* 1994; Yamauchi & Biswas 1997). However, the ability of the rice coleoptile to transport oxygen has not been shown experimentally and in wheat seedlings, the coleoptile actually impedes the movement of oxygen from the shoot to the root tip (Thomson & Armstrong 1990). Mesocotyl elongation brings the coleoptile node closer to the soil surface and is therefore concluded to increase the probability that the coleoptile will contact oxygen when a seedling germinates in an oxygen-depleted environment (Takahashi 1978; Yamauchi *et al.* 1994). Our data for red rice seem to contradict this conclusion because red rice mesocotyls were more abundant and longer under aerated conditions (Table 2.2). In our experiments, coleoptiles did not elongate into the air except when seedlings were started at 1.0 cm below the water surface. Therefore, the primary leaf was usually the first structure to emerge from the water. Primary leaves of rice have no blade or collar, just a sheath (Sylvester *et al.* 2001) which is the only part of the rice leaf that has aerenchyma with large intercellular spaces (Kaufman 1959; Matsukura *et al.* 2000). Primary leaves don't emerge from the rice embryo when oxygen is completely absent. Under hypoxic conditions however, the elongating primary leaf may more efficiently access and transport oxygen than the coleoptile. A bladeless, aerenchymatous primary leaf may be an adaptation that works in concert with the coleoptile and mesocotyl to facilitate rice seedling establishment in flooded soils.

CHAPTER 3. EFFECTS OF SUBMERGENCE AND HYPOXIA ON THE ANATOMY OF RICE SEEDLINGS

INTRODUCTION

Hypoxia and anoxia, the partial and complete depletion of environmental oxygen, develop in the soil and overlying water of flooded habitats because gas diffusion, which is 10,000 times slower in water than in air, does not compensate for the rapid biological consumption of dissolved oxygen (Ponnamperuma 1972; Gambrell *et al.* 1991). Although certain plant species (“wetland” plants) normally grow in flooded habitats, sustained growth and reproduction require that tissues throughout the plant receive oxygen for aerobic respiration, the main means of energy production (Vartapetian & Jackson 1997). Some wetland plants can grow with little to no oxygen during limited periods of their lifecycle such as when establishing a new generation or when responding to environmental changes. For example, coleoptile elongation in germinating seedlings of rice (Öpik 1973; Ishizawa *et al.* 1999) and some species of *Echinochloa* (Kennedy *et al.* 1980) can occur without oxygen. Rhizomes and tubers of a small number of species can survive periods of anoxia and some even produce shoots without oxygen (Barclay & Crawford 1982; Summers & Jackson 1994; Ishizawa *et al.* 1999). In response to submergence, many rice varieties and other wetland species exhibit rapid shoot elongation until contact with the atmosphere is restored (Jackson 1985; Voesenek *et al.* 1992). These examples of shoot elongation through oxygen-depleted soil and water are believed to enable the acquisition of oxygen directly from the atmosphere or via photosynthesis. Oxygen is transported from emergent tissues to roots, rhizomes, and submerged leaves via aerenchyma, a specialized tissue containing abundant, large gas spaces that are usually interconnected throughout a plant (for a recent review pertaining to aerenchyma see Jackson & Armstrong 1999). In addition to maintaining aerobic respiration within root tissues, transported oxygen diffuses out of roots into the rhizosphere where it is believed to further benefit the plant by oxidizing reduced phytotoxins such as hydrogen sulfide (Končalová 1990; Gambrell *et al.* 1991). The transport of oxygen and other gasses through aerenchyma gas spaces occurs by diffusion and/or mass flow. Gaseous metabolic products of plant and microorganismal metabolism, such as carbon dioxide, methane, and ethylene, are transported out of the root zone to the atmosphere via aerenchyma (Higuchi 1982; Higuchi *et al.* 1984; Butterbach-Bahl 1997; Visser *et al.* 1997). Considering how important accessing and transporting oxygen may be to rice seedlings that germinate while under water, we choose to investigate how oxygen concentration and shoot submergence affected aerenchyma development in rice seedlings that were between the germination and first leaf stages of development. Aerenchyma gas spaces were examined in the primary root, primary leaf and coleoptile, the first three structures to emerge from a germinating rice embryo.

Aerenchyma is either constitutive, meaning that it always forms during development, or inducible, meaning that it only forms in specific environmental conditions. Constitutive aerenchyma occurs most frequently in the shoots and roots of wetland species. Hypoxia induces root aerenchyma in some non-wetland species but is also associated with increased aerenchyma production in species that have constitutive aerenchyma (Smirnoff & Crawford 1983; Justin & Armstrong 1987). Rice and corn have respectively served as model species in studies of constitutive and of inducible root aerenchyma. Similarities and differences exist between root aerenchyma development in these species. Structural changes at the cellular and anatomical

levels during root aerenchyma formation have been described for corn (Campbell & Drew 1983; Jackson *et al.* 1985a). In rice, the general pattern of cell collapse to form root aerenchyma is consistent among studies (Justin & Armstrong 1991; Kang *et al.* 1998; Kawai *et al.* 1998; Samarajeewa *et al.* 1999). When aerenchyma formation is complete, strands of collapsed cortex cells adjoined at their tangential walls extend from the endodermis to the specialized outer cortex and epidermal layers (Clark & Harris 1981). When Kawai *et al.* (1998) studied aerenchyma development in primary rice roots using serial transverse sections, cell collapse began between 8 and 10 mm behind the root tip. In each radial file of cortex cells, the fifth cell from the endodermis was usually the first to collapse and was more expanded in the radial direction than the other cells. Cells at this position also stained with neutral red, indicating that vacuolar acidification may precede cell collapse. Using electron micrographs of tissues between six and 24 h-old, Webb and Jackson (1986) described the cellular events leading up to cortex cell collapse during rice root aerenchyma formation. At 6 h, cells occasionally had invaginations of the plasmalemma and middle lamella dissolution, at 12 h, cells displayed cytoplasmic changes, further detachment of the plasmalemma from the cell wall, cell wall degradation and decreased turgidity and by 24 h, many cortex cells had completely collapsed. This type of aerenchyma formation is referred to as lysigenous because cellular lysis, involving the collapse and death of cells, is believed to be the predominant process by which gas spaces form (Esau 1977). The gas space formation in rice root aerenchyma likely involves some schizogenous cell separation along the middle lamella of adjoining cells (Esau 1977; Webb & Jackson 1986; Kang *et al.* 1998).

Aerenchyma in rice leaves is also considered to be lysigenous. The primary leaf of rice seedlings develops as a sheath that lacks the blade and stipule displayed by all other “complete” leaves. The sheath is the part of the rice leaf that contains the largest and most abundant gas space cavities. The cylindrical gas spaces cavities are elongated along the longitudinal axis of the sheath and alternate with vascular bundles. In complete leaves, gas spaces in the blade are limited to the midrib region and decrease in number to zero about half way up the blade (Kaufman 1959). Leaf gas spaces are interrupted by uniformly spaced transverse septa/diaphragms that contain stellate parenchyma cells with large intercellular gas spaces and transverse veins (Kaufman, 1959; Matsukura *et al.*, 2000). Longitudinal gas space development in complete leaves occurs in a basipetal direction, starting in the blade and extending into the sheath. In the sheath, horizontal development of aerenchyma extends from the center outward. Sheath elongation occurs mainly at the base of this structure (Kaufman, 1959). So, elongation growth and aerenchyma development proceed in opposite directions within the leaf. We know of only one experiment that addressed the effects of flooding on rice leaf aerenchyma. Katayama’s (1960) study showed that in both the blade and sheath of the first three leaves of rice seedlings, gas space volume was greater in paddy-grown plants than in upland-grown plants.

The coleoptile of rice and other grass species is a small, short-lived, determinant structure. The rice coleoptile consists of an epidermal layer, relatively uniform mesophyll tissue and two vascular bundles that align with the longitudinal axis of the organ. Despite seemingly simple anatomy, there is spatial variation in cell length (personal observation), timing of senescence (Inada *et al.*, 1998), and rates of ethanol synthesis (Setter and Ella, 1994) within the rice coleoptile. Hypoxia is known to stimulate coleoptile elongation (Ranson and Parija 1955; Ohwaki 1967; Turner *et al.* 1981) but the effects of hypoxia on coleoptile aerenchyma are unknown.

In rice roots, the amount of aerenchyma gas space and the position of aerenchyma formation along the root have been shown to be influenced by flooding and/or hypoxia. However, some studies indicate that these variables do not appreciably affect constitutive amounts of rice root aerenchyma. For example, Jackson *et al.* (1985b) found that cortical gas space was greater in non-aerated rice roots than in aerated roots when comparing 1 d-old tissues but not 2 d- and 3 d-old tissues. When observing transverse sections from only a few positions along roots, John (1977) found no difference in gas space area between rice roots grown in aerated and nitrogen-flushed nutrient solutions. Two out of four rice varieties studied by Colmer *et al.* (1998) had significantly greater root porosity when grown in stagnant nutrient solution than when grown in aerated nutrient solution. Armstrong (1971) found that maximum root porosity occurred at the same distance behind the root tip (4.0-5.0 cm) in waterlogged and non-waterlogged rice roots. However, gas spaces initiated closer to the root apex (1.5-2.0 vs. 3-3.5 cm) and occupied a greater percent of root cross sections in waterlogged roots. Das and Jat (1977) reported that porosity was greatest in roots from rice plants that were continuously flooded compared to plants that were alternately flooded or not flooded. Primary rice root porosity varied significantly among seven rice varieties studied by Kludze *et al.* (1994). For each of these varieties, seedlings grown for 35 d in flooded soil had a higher root porosity than seedlings grown in drained soil. Prade and Trollenier (1990) found that rice root porosity was similar among plants grown in quartz silts with air-filled porosities ranging between 2 and 20% (v/v). However, root porosity increased if cellulose was added to silt with an air-filled porosity of 1.5 to 4% (v/v). Differences among these studies in variety, type of root, root age, method of measuring aerenchyma, and rooting medium likely confound conclusions about the effects of hypoxia on rice root aerenchyma. Additionally, in all of these studies, shoots were emergent while roots were either in soil or hydroponic solution. Given the constitutive system of aerenchyma gas space in rice, even if roots were in a hypoxic environment, oxygen could have been transported to roots from emergent shoots.

One objective of the current study was to clarify the effects of hypoxia on the anatomical development of primary rice roots especially when oxygen was not being supplemented by internal transport from the shoots. Aerenchyma was compared among emergent and completely submerged rice seedlings that grew in aerated and hypoxic water. The use of primary roots in this study reduced experimental variability because each population of roots was relatively uniform in age, growth rate, and nutrient status.

A second objective of this study was to determine the effects of hypoxia and submergence on primary leaf and coleoptile gas spaces. A single transverse section taken 2 to 3 mm above the embryo of each seedling was used when quantifying the area of gas space in the coleoptile and primary leaf. Little experimental attention has focused on how the anatomy of these structures is affected by environmental factors. Understanding how the development of gas spaces in primary leaves and coleoptiles is affected by the availability of oxygen is especially important because these organs are believed to initiate the acquisition and transport of oxygen when rice seedlings establish under water or in flooded soils (Kordan 1974a; Takahashi 1978; Yamauchi *et al.* 1994; Yamauchi & Biswas 1997; see also Chapter 2).

MATERIALS AND METHODS

Rice varieties

Two strains of rice, Lemont and straw hull red rice (red rice), were used in these experiments. The grain came from the same batches that were described in Chapter 2.

Hydroponic cultivation of seedlings and experimental design

Dehulled grains (“seeds”) of rice were attached to plastic embroidery screens using acetic acid-free silicone rubber sealant (GE RTV 128 translucent). Five Lemont and five red rice seeds were alternated 1.85 cm apart across the screens and oriented so that the coleoptile emerged from the top of the embryo during germination. Six screens were placed vertically over three diffusers (Sweetwater-brand, fine-pore 23.0 cm x 4.0 cm x 4.0 cm) in each of two 18 L plastic storage boxes filled with distilled, deionized water (Fig. 2.1). An inverted, 11.3 L plastic storage box was used as a loose-fitting lid for each 18 L box. Germination was initiated by placing seeds under one centimeter of water, in the dark, for 27.3 h. Then seeds, with swollen embryos, and in many cases, slightly elongated coleoptiles, were positioned 1.0, 2.5, and 4.5 cm below the water surface. “Aerated” boxes were continuously bubbled with air and “hypoxic” boxes were continuously bubbled with nitrogen gas. Bubbling was adjusted to be gentle and evenly distributed among the diffusers. Each day, screens were moved to different positions within the box and deionized water was added to replace evaporative loss. Water was completely replaced in hypoxic boxes on the 7th day of the experiment. Seedlings grew in a dark growth chamber (Environmental Growth Chambers, Chagrin Falls, OH) except for approximately 15 minutes per day when digital images were made.

Two 18 L boxes were used for the aerated treatment and two 18 L boxes were used for the hypoxic treatment. Aerated and hypoxic treatments were carried out consecutively because of space limitations within the growth chamber. For Lemont and red rice, there were initially 20 seedlings positioned at each of the three depths in aerated water (aerated-1.0 cm, aerated-2.5 cm and aerated-4.5 cm treatments) and 20 seedlings positioned at each of the three depths in hypoxic water (hypoxia-1.0 cm, hypoxia-2.5 cm and hypoxia-4.5 cm treatments). Lemont and red rice seedlings at each depth were distributed evenly between the two boxes. Water temperature, dissolved oxygen concentration and pH were measured daily. Water temperature was 27.3 ± 1.3 °C in the aerated boxes and 28.6 ± 0.6 °C in the hypoxic boxes. Dissolved oxygen concentration, measured with a dissolved oxygen instrument (YSI 85), averaged 8.02 ± 0.45 mg/L in the aerated boxes and 0.94 ± 0.26 mg/L oxygen in the hypoxic boxes. The pH of the water, measured with a pH electrode, averaged 7.2 in both the aerated and hypoxic boxes.

The germinated Lemont and red rice seeds were positioned at the three depths to create brief, intermediate and continuous durations of leaf submergence prior to harvest. Seedlings from the aerated-2.5 cm and hypoxia-2.5 cm treatments were not used for anatomical study but confirmed the correlation between leaf emergence from hypoxic water and increased root elongation rates that occurred in a previous study (Chapter 2).

Seedlings from the aerated and hypoxic treatments grew at different rates and were therefore harvested after 5 d and 10 d, respectively. At the time of harvest, each healthy seedling was

placed in a 10 ml centrifuge tube containing FAA fixative (formalin, glacial acetic acid, ethanol, deionized water (2:1:10:7, v/v)).

Data Collection and Analysis

Growth measurements Digital images of the seedlings were collected with a flat bed scanner that was attached to a desktop computer (Fig. 2.2). Seedlings were scanned approximately every 24 h, starting 16 to 19 h after bubbling with air or nitrogen gas began. For each seedling, the lengths of the primary root, coleoptile, primary leaf and second leaf were measured from the scanned images using Adobe Photoshop 5.0 (Fig. 2.3). Primary root length was measured from the root-embryo junction to the root tip. Coleoptile length was measured from the middle of the coleoptile node to the tip of the coleoptile; if no mesocotyl was present the coleoptile node was at the shoot-embryo junction. Primary leaf and second leaf lengths were measured from the shoot-embryo junction to the tip of the leaf. The primary leaf and the second leaf that grows through the primary leaf were not always distinguishable from each other in the scanned images. Therefore, a distinction between the two leaf lengths was made only when measuring the final scanned images taken just prior to harvest. The mean maximum coleoptile lengths were calculated using measurements of aerated seedlings that were between 117 and 119 h-old and of hypoxically- treated seedlings that were between 212 and 213 h-old.

For each seedling, lengths of primary roots, leaves and coleoptiles were plotted as a function of time. Using all healthy seedlings, the mean daily lengths of these structures were also calculated for each treatment and plotted as a function of time. Four seedlings from each of the eight treatments were chosen for anatomical analysis. These 32 seedlings were selected because they met the desired submergence conditions and because their individual root lengths were close to mean root length values throughout the growth period. Mean lengths of primary roots, primary leaves, second leaves and coleoptiles were then calculated for each treatment using the four replicate seedlings that were chosen for anatomical analysis.

Anatomy Hand sections were viewed with a Nikon Microphot light microscope and photographed using a SPOT RT digital camera attached to the microscope. Areas were calculated from the digital images. Each area was traced and filled using Adobe Photoshop 5.0. The number of pixels within each filled area was converted to area in μm^2 .

Shoots Three transverse sections (0.25 to 0.33 mm thick) were taken between 2 and 3 mm above the coleoptile node of each seedling. The coleoptile, primary leaf and any other leaves that began to form during the growth period were visible in each transverse section. The following areas were determined from one of the three transverse sections from each seedling: total coleoptile area, total area of coleoptile gas spaces, total primary leaf area, total area of primary leaf gas spaces (Fig. 3.1). These areas were used to calculate the percent of coleoptile area occupied by gas space and percent of the leaf area occupied by gas space in each section. Sections from four replicate seedlings were used to calculate mean values for each of the eight treatments.

Coleoptiles were excised from the shoots after the cross sections were taken and then splayed onto a glass microscope slide with the adaxial (inner) epidermal surface facing upward. Epidermal cells were viewed with the light microscope adjusted for differential interference

contrast optics, digitally photographed and then measured using Adobe Photoshop 5.0. The epidermal cells that were measured were located between the two vascular bundles in the region of the coleoptile where the two vascular bundles began to taper towards each other. A mean epidermal cell length and a mean epidermal cell width were determined for each coleoptile using measurements of 10 epidermal cells. The mean cell length and mean cell width of individual coleoptiles were used to calculate mean values for each of the eight treatments. Mean cell lengths for each treatment were plotted against mean coleoptile lengths for each treatment. A correlation coefficient was determined for the pooled data from all treatments.

Roots Transverse sections were made along the entire length of each of the 32 roots. For each root, cross sections (approx. 0.11 mm thick) were taken at 0.5 mm and 1.0 mm behind the root tip and thereafter at intervals ranging from one to 10 mm. Intervals were closer in the region of the root where aerenchyma gas spaces were most actively forming. For each seedling, daily root elongation rates were determined and used to estimate tissue age at each given distance behind the root tip.

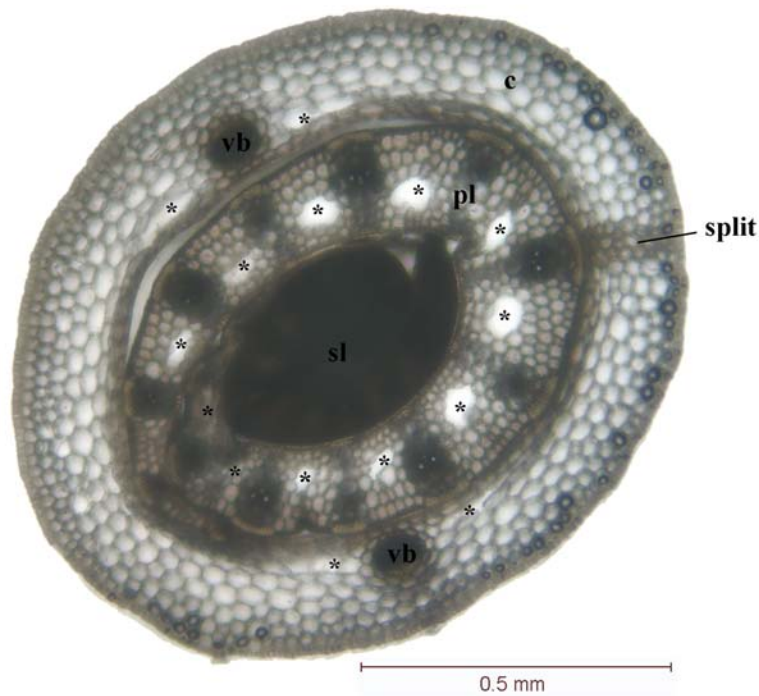
One section at each distance behind the root tip was analyzed for each root. Areas of the following were determined for each transverse section: 1) the total section, 2) the stele plus the endodermis (“stele”), 3) the cortex layers that have the potential to form aerenchyma (“cortex”), 4) the aerenchyma gas space within the cortex and 5) the epidermis and specialized outer cortex layers (“outer layers”) (Fig. 3.2). Each transverse section was assumed to be a circle so that a radius could be calculated from the total area. The portion of the radius extending from the center of the section to the outer edges of the stele is referred to as the thickness of the stele (Fig. 3.2). The portion of the radius extending from the outer edges of the stele to the outer edges of cortex is referred to as the thickness of the cortex. And, the portion of the radius extending from the outer edges of cortex to the outside of the section is referred to as the thickness of the outer layers. The percent of the cortex area occupied by gas space was also determined for each section. For each treatment, mean radius length, mean stele, cortex and outer layer thicknesses, mean gas space percentage and mean tissue age at each distance behind the root tip were calculated using sections from four replicate seedlings.

RESULTS

Morphology of seedlings

The anatomy of seedlings started at 1.0 and 4.5 cm below the water surface was studied. Mean final lengths of primary roots, leaves and coleoptiles for these seedlings are presented in Table I. The growth curves of these seedlings were similar to those in Chapter 2 and are presented in Appendix 1. Seedlings of Lemont and red rice from the aerated-1.0 cm and hypoxia-1.0 cm treatments were termed “emergent” because most root and leaf elongation occurred while leaves were in contact with the atmosphere. Lemont seedlings from the aerated-4.5 cm treatment and Lemont and red rice seedlings from the hypoxia-4.5 cm treatments were termed “submerged” because they remained completely under water until harvest. Red rice seedlings from the aerated-4.5 cm treatment are termed “semi-emergent” because their leaves emerged from the water during the last 26 h of growth.

A.



B.

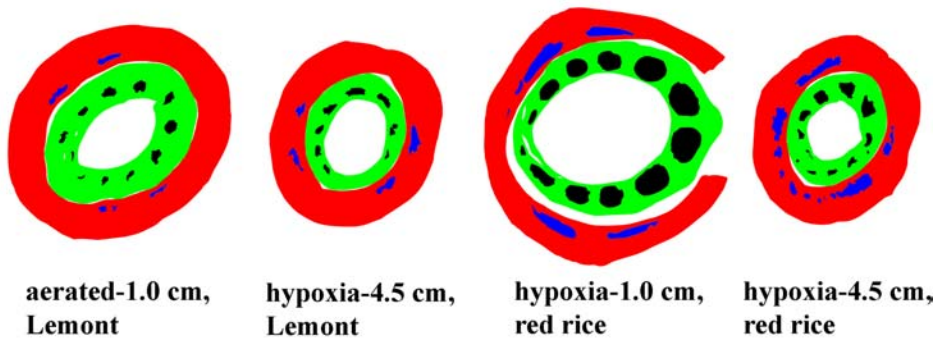


Figure 3.1 A representative transverse shoot section (A), taken between 2 and 3 mm above the seed, includes the coleoptile (c), primary leaf (pl) and second leaf (sl). In some treatments, coleoptiles split open along the region of dark-staining cells (split). The following areas were determined for each transverse shoot section: total coleoptile area, total area of coleoptile gas spaces (*), total primary leaf area, total area of primary leaf gas spaces (*). Each area was traced and filled using Adobe Photoshop 5.0. The number of pixels within each filled area was converted to area in μm^2 . The percent of coleoptile area occupied by gas space and the percent of leaf area occupied by gas space were also determined. Representative traced images (B) show variation among some of the treatments. The far-left traced image was made using the transverse section in A.

For both Lemont and red rice, lengths of primary roots were similar among seedlings from the aerated-1.0 cm, aerated-4.5 cm, and hypoxia-1.0 cm treatments (Table I). Lengths of second leaves were similar among the seedlings from the aerated-1.0 cm and aerated-4.5 cm treatments but longer in seedlings from the hypoxia-1.0 cm treatment (Table I). In these three treatments, red rice had longer primary roots and second leaves than Lemont. Seedlings from the hypoxia-4.5 cm treatment had much shorter primary roots than seedlings from the other three treatments. Primary root and second leaf lengths were similar between Lemont and red rice seedlings from the hypoxia-4.5 cm treatment (Table I).

Table 3.1 Mean final lengths (± 1 S.E.) of primary roots and second leaves of Lemont and red rice seedlings grown in aerated and hypoxic water. Germinated seeds were positioned at either 1.0 cm or 4.5 cm below the water surface so that as seedlings grew, leaves either emerged from the water or remained submerged until harvest. The time of harvest was about 118 h in aerated treatments and about 236 h in hypoxia treatments. Each mean was calculated using the four replicate seedlings that were used in anatomical analysis.

treatment	submergence status		root length (cm)		2 nd leaf length (cm)	
	Lemont	red rice	Lemont	red rice	Lemont	red rice
aerated-1.0 cm	emergent	emergent	9.93 \pm 0.07	12.41 \pm 0.18	4.19 \pm 0.20	6.27 \pm 0.12
hypoxia-1.0 cm	emergent	emergent	9.61 \pm 0.11	11.30 \pm 0.26	7.69 \pm 0.17	10.99 \pm 0.41
aerated-4.5 cm	submerged	semi-emergent	9.66 \pm 0.11	11.61 \pm 0.17	4.54 \pm 0.22	6.36 \pm 0.35
hypoxia-4.5 cm	submerged	submerged	2.20 \pm 0.07	1.83 \pm 0.07	3.74 \pm 0.09	3.78 \pm 0.26

In Lemont, the mean length of primary leaves in the hypoxia-4.5 cm treatment was 34 to 61 % longer than mean primary leaf lengths in the other three treatments (Fig. 3.3). In both aerated and hypoxia treatments, primary leaves of red rice were longest when seedlings were initially positioned at 4.5 cm below the water surface. When Lemont and red rice seedlings from the hypoxia-4.5 cm treatment were harvested, the second leaf was only about 1 mm longer than the primary leaf.

Within each treatment, red rice seedlings had a greater mean coleoptile length than Lemont seedlings (Fig. 3.3). Within the aerated and hypoxia treatments, Lemont and red rice seedlings started at 4.5 cm had a longer mean coleoptile length than seedlings started at 1.0 cm. Lemont coleoptiles were longer in the hypoxia treatments than in the aerated treatments. In contrast, red rice coleoptiles from the aerated-4.5 cm treatment were longer than red rice coleoptiles from the hypoxia-1.0 cm treatment (Fig. 3.3).

Root anatomy

Root hairs In roots from the aerated and hypoxia-1.0 cm treatments, root hairs were usually absent at 1mm behind the root tip and present at 4 mm behind the root tip. The position where root hairs formed varied among roots from the hypoxia-4.5 cm treatment.

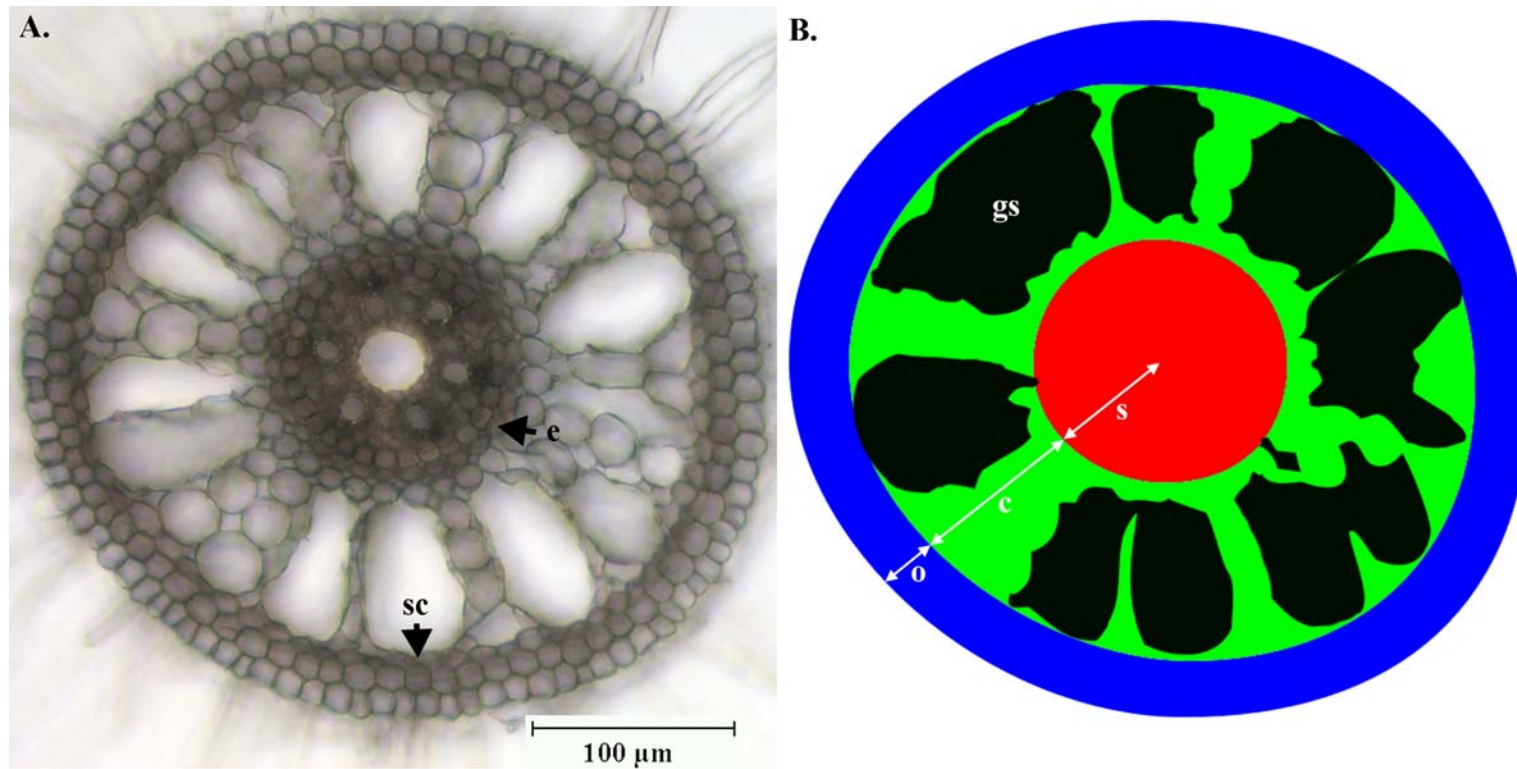


Figure 3.2 Representative transverse root section (A) for which the following areas were determined (B): the “outer layers” (o) which include the epidermis and specialized outer cortex layers, the “cortex” (c) which includes only the cortex layers that form aerenchyma, the aerenchyma gas space (gs) and, the “stele” (s) which includes the stele and endodermis. Each area was traced and filled using Adobe Photoshop 5.0. The number of pixels within each filled area was converted to area in μm^2 . The percent of cortex area occupied by gas space was also determined for each section. Each transverse section was assumed to be a circle so that a radius could be calculated from the total area. The portion of the radius extending from the center of the section to the outer edges of the stele is referred to as stele thickness. The portion of the radius extending from the outer edges of the stele to the outer edges of cortex is referred to as the cortex thickness. And, the portion of the radius extending from the outer edges of cortex to the outside of the section is referred to as the thickness of the outer layers. The endodermis (e) and sclerified outer cortex layer (sc) are indicated in A.

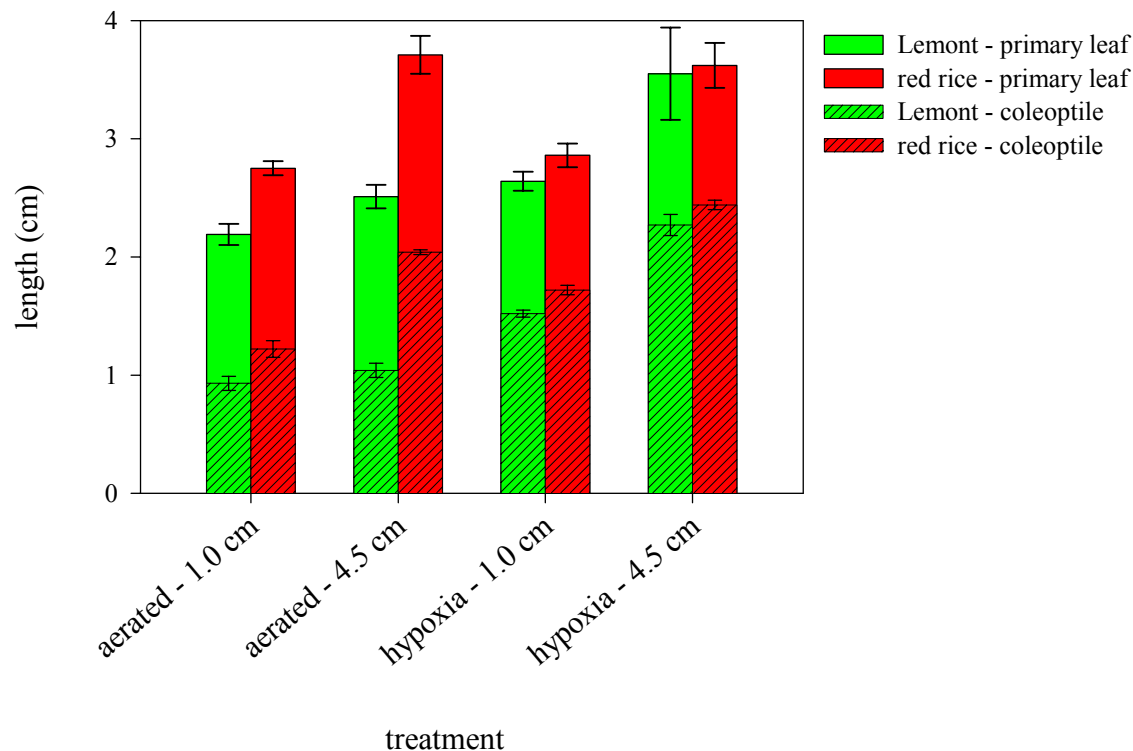


Figure 3.3 Mean final lengths of coleoptiles and primary leaves of Lemont and red rice seedlings grown in aerated and hypoxic water. Germinated seeds were positioned at either 1.0 cm or 4.5 cm below the water surface so that as seedlings grew, leaves either emerged from the water or remained submerged. Each mean (± 1 S.E.) was calculated using the four replicate seedlings that were used in anatomical analysis.

Root radius In each section, the root radius was the sum of the thicknesses of the stele, cortex and outer layers (Fig. 3.2). Data for root radii and for thicknesses of the stele, cortex and outer layers are presented in Appendix 2. Compared to the stele and outer layers, the cortex had the greatest thickness which fluctuated more along the length of the roots. Therefore, changes in root radii along the length of the roots most closely paralleled changes in cortex thickness.

Arrangement of cortex cells in transverse root sections Figure 3.4 allows comparison among transverse sections from three different roots. Sections were taken 91 mm (Fig. 3.4A) and 4 mm (Fig. 3.4D) behind the root tip of a Lemont root from the aerated-1.0 cm treatment that was 99 mm in length, 18 mm (Fig. 3.4B) and 2 mm (Fig. 3.4E) behind the root tip of a red rice root from the hypoxia-4.5 cm treatment that was 20 mm in length, and 96 mm (Fig. 3.4C) and 36 mm (Fig. 3.4F) behind the root tip of a red rice root from the hypoxia-1.0 cm treatment that was 106 mm in length. Transverse sections of roots from the hypoxia-4.5 cm treatment revealed that there was a difference in the diameter of the root between the root tip and the seed (Fig. 3.4B & E).

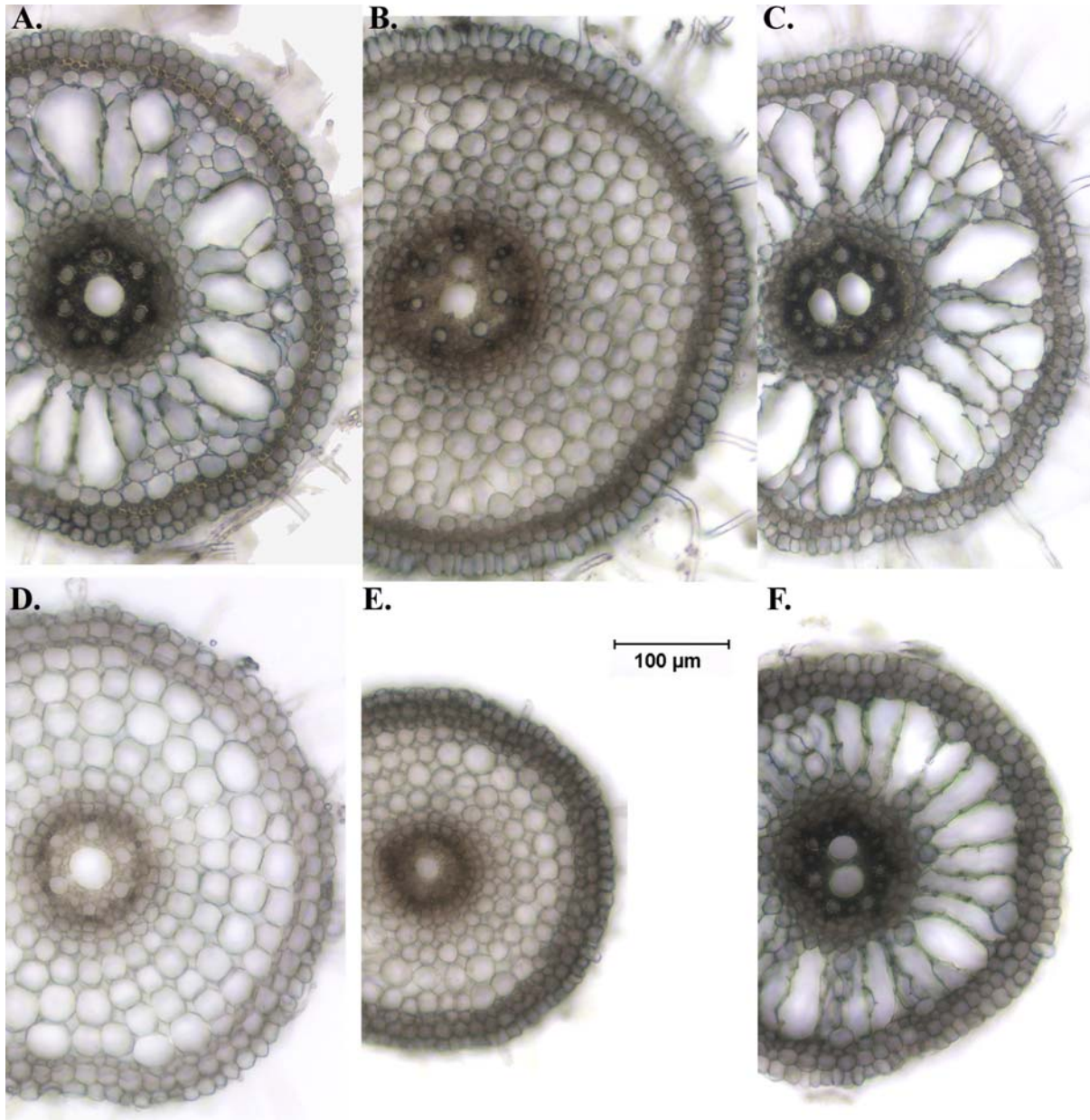
Aerenchyma gas space was present in all sections except those that were taken relatively close to the root tip where gas spaces had not yet formed. In transverse sections taken close to the root tip, cortex cells that would eventually become aerenchyma usually formed four concentric layers between the sclerified outer cortex layer and the endodermis (Fig. 3.4D & E). Cells in neighboring concentric layers were aligned so that they formed radial files consisting of four evenly stacked cells. Each cortex cell was usually surrounded by four intercellular spaces. There was some variation in this cortex cell arrangement. For example, sometimes partial concentric layers, extending only a portion of the way around the circumference of the root, formed just inside the sclerified outer cortex layer. In this circumstance, some of the radial files had five, rather than four, stacked cortex cells. Anticlinal cell divisions in the outer two concentric layers often caused the radial files to branch once towards the outside of the cortex. Among transverse sections taken close to the root tip, root diameter and the size of cortex cells were smallest in sections from the hypoxia-4.5 cm treatment (Fig. 3.4E).

Serial transverse sections indicated that the cortex cell arrangement existing close to the root tip extended into the portions of the root where aerenchyma gas spaces were most actively forming. Each radial file of cells formed prior to aerenchyma gas space formation appeared to result in a radius of collapsed cells between the sclerified outer cortex layer and the endodermis (Fig. 3.4F). Similarly, each singly-branched radial file of cells appeared to result in a singly-branched “radius” between the sclerified outer cortex layer and the endodermis (Fig. 3.4F).

The cortex cell arrangement in transverse sections taken close to the seed differed from the cortex cell arrangement in transverse sections taken close to the root tip. In sections taken from points along the root where aerenchyma formation was complete (i.e. near the seed), the cortex cell arrangement that existed prior to gas space formation was deduced from the patterns formed by collapsed and un-collapsed cells. The transverse root sections taken close to the seed (Fig. 3.4A, B & C) appeared to have a greater number of cortex cells than sections taken near the root tip (Fig. 3.4 D & E). And, cortex cells close to the seed were not always arranged in concentric layers, were not consistently aligned in orderly radial files and frequently had more than four intercellular spaces surrounding them. This cell arrangement is most noticeable in Figure 3.4B because not much aerenchyma gas space formed in this root from the hypoxia-4.5 cm treatment. In transverse sections of roots from the other treatments, especially those that were aerated, cells in the outer cortex layers often did not collapse during aerenchyma gas space formation (Fig. 3.4A). The number of intercellular spaces among these un-collapsed cortex cells was often greater than four. The pattern of collapsed cells in Figure 3.4C appeared web-like because, prior to cell collapse, there had been few un-branched or singly-branched radial cell files extending between the sclerified outer cortex layer and the endodermis.

The pattern of collapsed and un-collapsed cortex cells in serial transverse root sections indicated that as distance from the seed increased, cells within the cortex gradually decreased in number and became organized into the arrangement that occurred near the root tip and where aerenchyma was actively forming. Moving away from the seed, complete transition in cell arrangement was inferred to have occurred when there were few un-collapsed cells along the outer cortex and a uniform pattern of collapsed cells. The location of this transition along the root varied among treatments and sometimes among roots from the same treatment (Fig. 3.5 & 3.6).

Figure 3.4 Representative transverse root sections from three roots showing different arrangements of collapsed and un-collapsed cortex cells. Sections were from 91 mm (A) and 4 mm (D) behind the tip of a Lemont root from the aerated-1.0 cm treatment that was 99 mm long, 18 mm (B) and 2 mm (E) behind the tip of a red rice root from the hypoxia-4.5 cm treatment that was 20 mm long, and 96 mm (C) and 36 mm (F) behind the tip of a red rice root from the hypoxia-1.0 cm treatment that was 106 mm long. Close to the root tip (D, E), cortex cells that would eventually produce aerenchyma usually formed radial files of four cells that had four intercellular spaces surrounding them. Patterns of collapsed and un-collapsed cortex cells indicated that sections taken close to the seed (A, B & C) had a greater number of cortex cells than sections further away from the seed. Cortex cells near the seed did not consistently form radial files and frequently had more than four intercellular spaces surrounding them. This is most noticeable in (B) because not much gas space formed in this section from the hypoxia-4.5 cm treatment. The pattern formed by collapsed cortex cells was more uniform in sections taken closer to the root tip (F) because each radial file of cells resulted in a radius of collapsed cells. Nearer to the seed (A, C), the pattern formed after cortex cells collapsed was less uniform because there were fewer un-branched radial files of cortex cells prior to gas space formation. Also near the seed, cells in the outer cortex layers often remained un-collapsed (A).



Aerenchyma formation in aerated roots The change in cortex gas space (the percent of cortex area occupied by gas space) as distance behind the root tip increased was similar among all aerated roots (Fig. 3.5). At 13 mm behind the root tip, mean cortex gas space was between 1 and 4 % among all aerated Lemont and red rice roots. Beyond 13 mm, mean cortex gas space increased to a maximum that occurred at 56 mm behind the root tip in all aerated Lemont roots (Fig. 3.5A), at 56 mm behind the root tip in aerated-4.5 cm red rice roots and at 66 mm behind the root tip in aerated-1.0 cm red rice roots (Fig. 3.5B). Among aerated Lemont and red rice roots, the maximum mean cortex gas space occurred in tissues that had a mean age of 40 to 47 h (Fig. 3.5C & D). The maximum mean cortex gas space in aerated red rice roots was about 12 % larger than the maximum mean cortex gas space in aerated Lemont roots (Fig. 3.5A & B). There was fluctuation in the mean cortex gas space between the position of maximum cortex gas space and the seed in all aerated roots.

Aerenchyma formation in roots from hypoxia treatments In Lemont and red rice roots from the hypoxia-1.0 cm treatment, the mean cortex gas space was 8 to 9 % at 4 mm behind the root tip and increased most steeply between 4 and 19 mm behind the root tip (Fig. 3.6A & B). Between 19 and 36 mm behind the root tip, mean cortex gas space rose more gradually to a maximum of about 90 % in both Lemont and red rice. Between 60 mm and the seed, the mean amount of cortex gas space declined gradually. Mean cortex gas space in sections that were nearest to the seed was 79 % of the maximum in Lemont and 83 % of the maximum in red rice.

In Lemont and red rice, roots from the hypoxia-1.0 cm and hypoxia-4.5 cm treatments had a similar increase in mean cortex gas space between 0.5 and 10 mm behind the root tip (Fig. 3.6A & B). However, in the hypoxia-4.5 cm treatment, the maximum mean cortex gas space occurred at 16 mm behind the root tip in Lemont and at 7 mm behind the root tip in red rice. Maximum mean cortex gas space in roots from the hypoxia-4.5 cm treatment was 41% greater in red rice than in Lemont. In Lemont, the mean cortex gas space decreased by 26 % between 16 mm and 19 mm, the position closest to the seed. In red rice, the mean cortex gas space decreased by 49 % between 7 mm behind the root tip and the position closest to the seed.

In both Lemont and red rice, the relationship between cortex gas space and tissue age differed between the hypoxia-1.0 cm and hypoxia-4.5 cm treatments (Fig. 3.6C & D). Between the tissue ages of 0 and 60 h, mean cortex gas space increased more rapidly in roots from the hypoxia-1.0 cm treatment than roots from the hypoxia-4.5 cm treatment. At all tissue ages, roots from the hypoxia-1.0 cm treatment had more cortex gas space than roots from the hypoxia-4.5 cm treatment. In Lemont, the maximum mean cortex gas space was 57% smaller in roots from the hypoxia-4.5 cm treatment compared to roots from the hypoxia-1.0 cm treatment. In red rice, the maximum mean cortex gas space was 39 % smaller in roots from the hypoxia-4.5 cm treatment compared to roots from the hypoxia-1.0 cm treatment. For both Lemont and red rice, the mean cortex gas space in sections closest to the seed was about 60 % smaller in the hypoxia-4.5 cm treatment compared to the hypoxia-1.0 cm treatment.

Shoot anatomy

Primary leaves In both Lemont and red rice, the mean primary leaf area in transverse sections taken from between 2 and 3 mm above the coleoptile node was greater in the aerated-1.0 cm treatment than in the aerated-4.5 cm treatment and also greater in the hypoxia-1.0 cm treatment

than in the hypoxia-4.5 cm treatment (Fig. 3.7A). The greatest mean primary leaf areas for Lemont and red rice occurred in the hypoxia-1.0 cm treatment. Mean primary leaf areas in the hypoxia-4.5 cm treatment were similar between Lemont and red rice and were the smallest among all treatments.

In the transverse sections of primary leaves of Lemont and red rice, the mean number of gas space cavities was greater in the hypoxia-1.0 cm treatments compared to the other treatments (Fig. 3.7B). The one to two additional gas space cavities in transverse sections from the hypoxia-1.0 cm treatments were located at the outer edges of the leaf and were small relative to the other gas cavities within the leaf (Fig. 3.2). The transverse sections of leaves from the hypoxia-1.0 cm treatment had the largest mean percent of leaf area occupied by gas space (Fig. 3.7C).

Coleoptiles The mean final length of coleoptiles was highly correlated ($r = 0.978$) with the mean length of adaxial epidermal cells when data from all treatments was pooled (Fig. 3.8).

Transverse sections of cylindrical coleoptiles enclosed transverse sections of the primary leaf and second leaf (Fig. 3.2). Mesophyll tissue in the transverse coleoptile sections was composed of circular cells that tended to have a smaller diameter towards the abaxial epidermis. In some treatments, the mesophyll tissue appeared compacted presumably due to the pressure applied to the coleoptile when the enclosed leaves expanded. Each coleoptile had two vascular bundles that were on opposite sides of the transverse section. When a transverse section was bisected by a line connecting the vascular bundles, the midpoint of one half (the split-half) of the coleoptile had a region of mesophyll cells that stained dark when fixed. This region, extending between the abaxial and adaxial epidermal layers, was where the coleoptile split open due to continued leaf expansion. Split coleoptiles were always present in the hypoxia-1.0 cm treatments, 50 to 75 % present in the aerated-1.0 cm and aerated-4.5 cm treatments and never present in the hypoxia-4.5 cm treatment. When present, gas spaces in the transverse coleoptile sections were located close to the adaxial surface and next to the vascular bundles (Fig.1). When more extensive, gas spaces mostly expanded parallel to the epidermal layers. Gas spaces formed more frequently and had a greater area on the non-split half of the coleoptile (data not shown).

The mean coleoptile area in transverse sections did not vary greatly among all treatments; the largest mean coleoptile area was 25 % larger than the smallest mean coleoptile area (Fig. 3.9A).

Transverse sections of Lemont coleoptiles from the hypoxia-1.0 cm treatment had a 1.4 to 3.7 times larger mean percent of coleoptile area occupied by gas space than Lemont coleoptiles from the other treatments (Fig. 3.9B). Red rice coleoptiles from the hypoxia treatments had a 3.4 to 12.3 times larger mean percent of coleoptile area occupied by gas space than red rice coleoptiles from the aerated treatments. Red rice coleoptiles had more gas space than Lemont coleoptiles in all but the aerated-1.0 cm treatments.

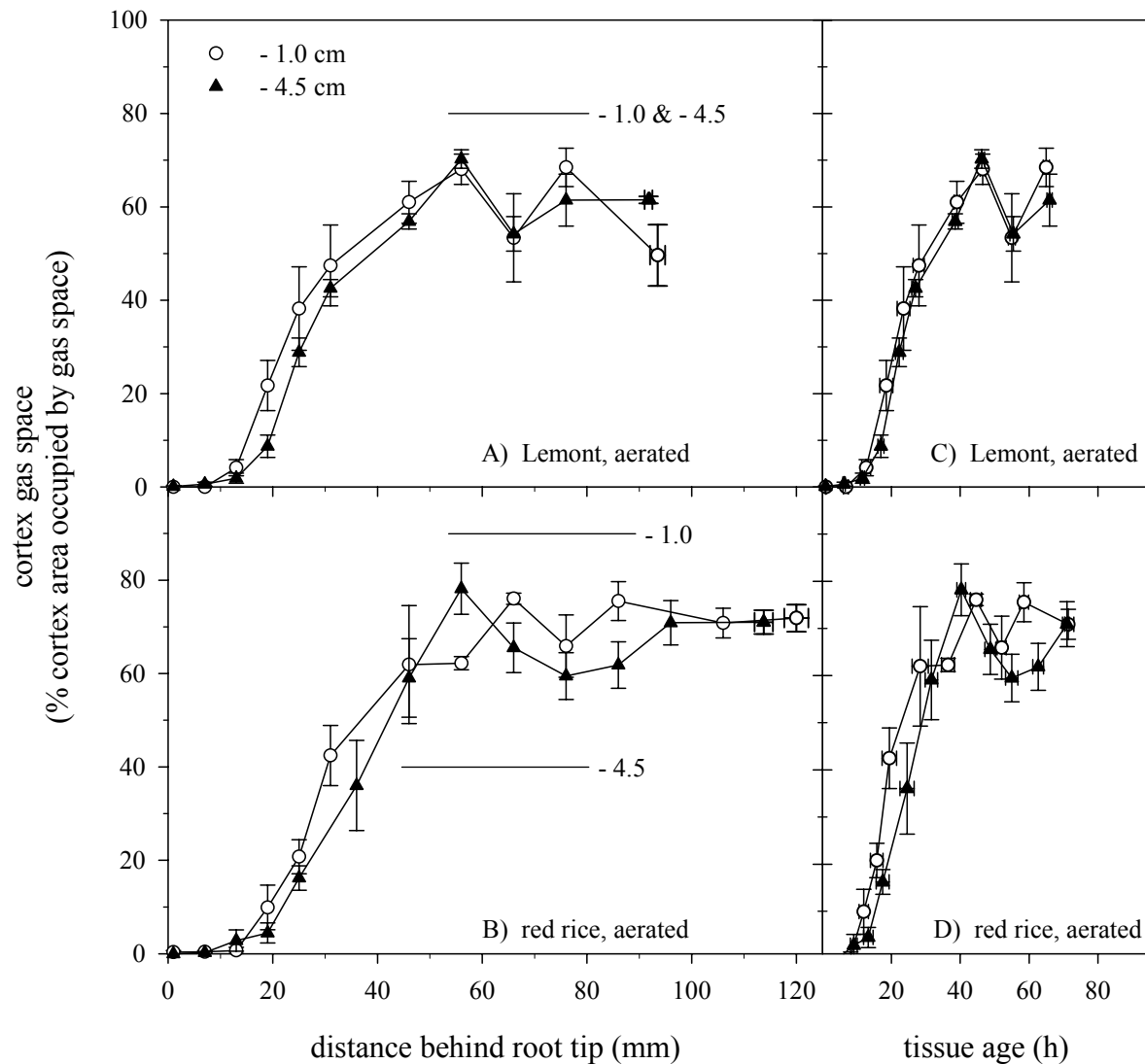
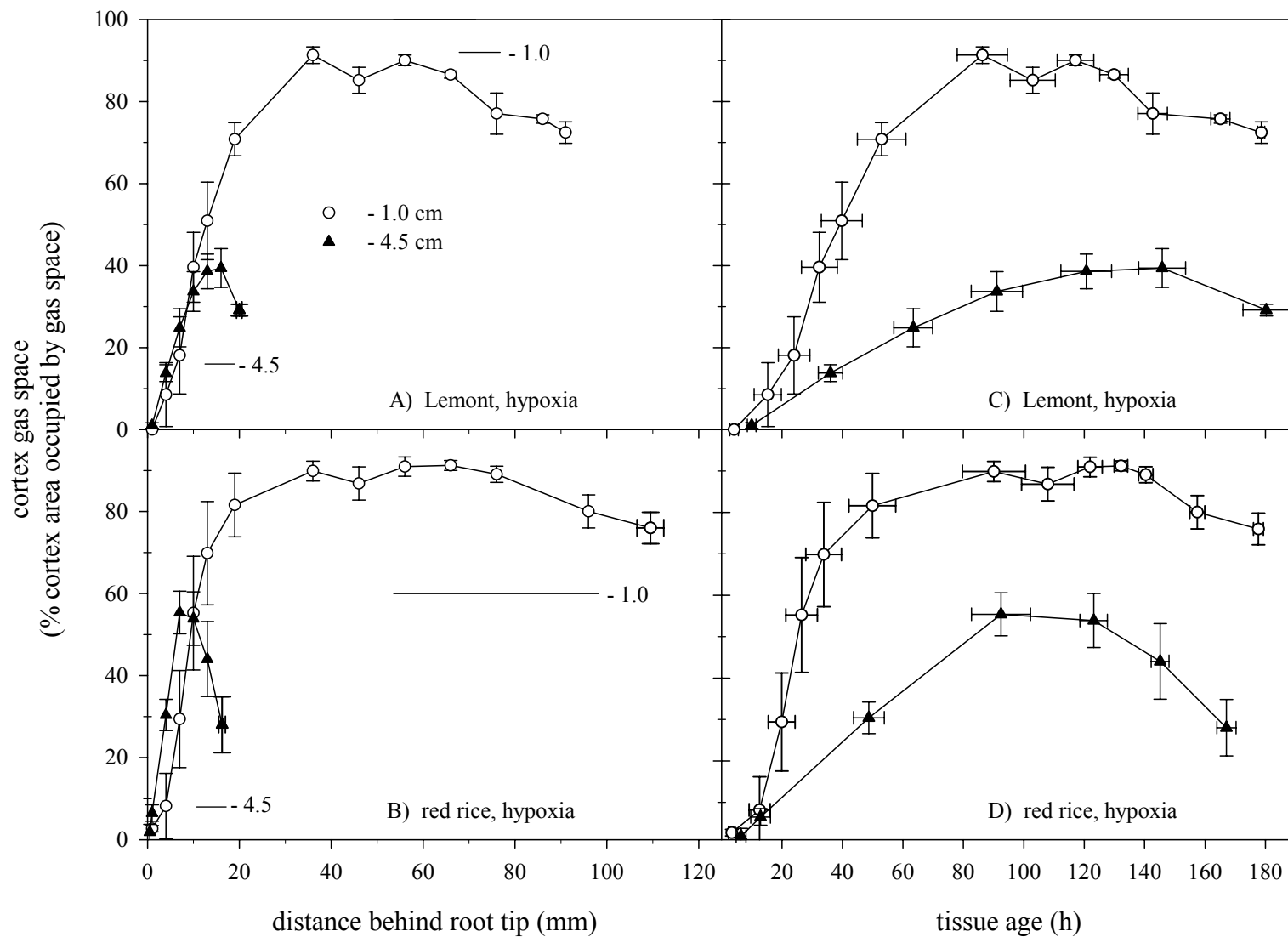


Figure 3.5 Percent of cortex occupied by gas space in root cross sections from Lemont (A and C) and red rice seedlings (B and D) grown in aerated water. Data are presented as a function of distance behind the root tip (A and B) and of tissue age (C and D). Germinated seeds were positioned at either 1.0 cm (circles) or 4.5 cm (triangles) below the water surface so that as seedlings grew, leaves either emerged from the water or remained submerged. Values are means (± 1 S.E.) of four replicate root sections taken at the same distance behind the root tips of four different root. The position of the transition between the two cortex cell arrangements is indicated for each treatment in A and B

Figure 3.6 Percent of cortex occupied by gas space in root cross sections from Lemont (A and C) and red rice (B and D) seedlings grown in hypoxic water. Data are presented as a function of distance behind the root tip (A and B) and of tissue age (C and D). Germinated seeds were positioned at either 1.0 cm (circles) or 4.5 cm (triangles) below the water surface so that as seedlings grew, leaves either emerged from the water or remained submerged. Values are means (± 1 S.E.) of four replicate root sections taken at the same distance behind the root tips of four different roots. The position of the transition between the two cortex cell arrangements is indicated for each treatment in A and B.



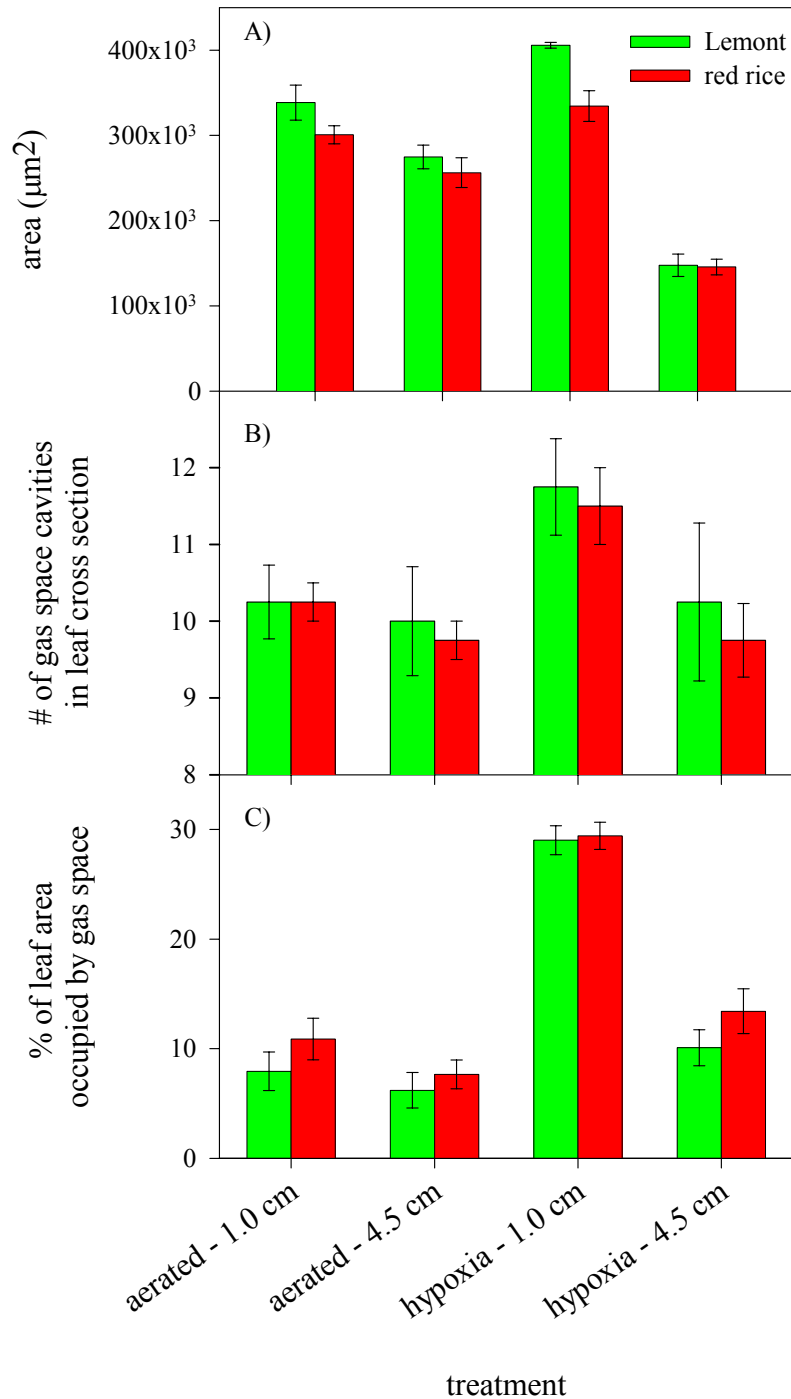


Figure 3.7 Area (A), number of gas space cavities (B) and percent of area occupied by gas space (C) for transverse sections of primary leaves taken between two and three mm above the coleoptile node in Lemont and red rice seedlings grown in aerated and hypoxic water. Germinated seeds were positioned at either 1.0 cm or 4.5 cm below the water surface so that as seedlings grew, leaves either emerged from the water or remained submerged. Values are means (± 1 S.E.) of four replicate leaf cross sections taken from four different seedlings.

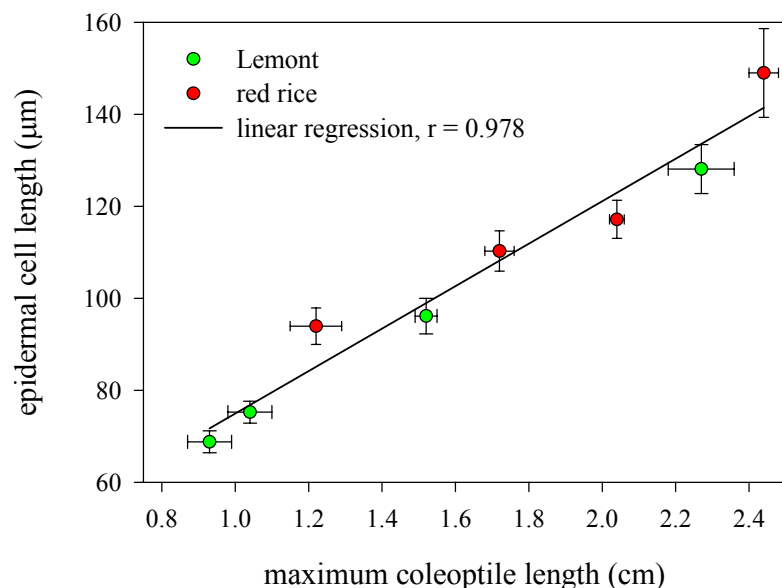


Figure 3.8 Adaxial epidermal cell length vs. maximum coleoptile length. A linear regression was fit to data pooled from all treatments. Values are means (± 1 S.E.) of four replicate coleoptiles.

DISCUSSION

Primary roots

Complete leaf submergence presumably prevented internal oxygen transport from the leaves to the root tips of seedlings in this experiment. Final mean lengths of primary roots were similar between emergent and submerged seedlings that grew in aerated water (Table 3.1). Seedlings that remained submerged in hypoxic water had relatively short primary roots compared to seedlings that grew in hypoxic water with emergent leaves. Therefore, as in Chapter 2, internal oxygen transport was concluded to be important for root growth in hypoxic but not aerated water. Primary roots of seedlings that grew in hypoxic water with emergent leaves were similar in length to, but 118 h older than, the primary roots of aerated seedlings. Therefore, even when leaves were emergent, root growth was slower in hypoxic water than in aerated water. The effects of hypoxia upon aerenchyma in primary rice roots was also found to be dependent upon whether or not oxygen transport was facilitated by leaf contact with the atmosphere. Differences in aerenchyma development between Lemont and red rice roots were small compared to differences among aerated roots and roots from the hypoxia treatments.

Aerenchyma formation Root elongation results from cell division and cell elongation in the root tip region. Fully expanded root hairs at a given position behind the root tip indicate that, at that position, most tissues within the root have ceased to elongate and begun to differentiate and mature (Taiz and Zeiger 1998). In roots from the aerated and hypoxia-1.0 cm treatments, root hairs were usually absent at 1 mm behind the root tip and present at 4 mm behind the root tip.

Therefore, the completion of cell elongation was estimated to have occurred somewhere between 1 and 4 mm behind the root tip. Considering the relative positions of cell collapse and root hairs along roots from the aerated and hypoxia-1.0 cm treatments, we concluded that cortex cell collapse to form aerenchyma gas spaces began to occur after the cessation of cell elongation. This agrees with the findings of Samarajeewa *et al.* (1999) who investigated the effect of salinity on aerenchyma development in primary rice roots. Interestingly, lysigenous gas space formation and cell division occurred simultaneously in the first 40 mm behind the root tips of *Sagittaria lancifolia* roots (Schussler & Longstreth 1996). In roots from the hypoxia-4.5 cm treatment, there was variability in the position of root hair formation probably because hypoxia interfered with normal cell growth and differentiation when oxygen transport to the root tip was prevented.

In aerated roots of Lemont and red rice, cortex gas space increased from a minimum to a maximum between 10 and 56 mm behind the root tip (Fig. 3.5A & B). This is consistent with gas space development in the waterlogged, rather than non-waterlogged, rice roots studied by Armstrong (1971). The maximum mean cortex gas space of aerated Lemont roots was approximately 10 % smaller than that of aerated red rice roots. Between 56 mm and the seed, cortex gas space in both varieties never fluctuated lower than 73% of the maximum. So, the process of gas space formation, progressing back from the root tip, occupied over half the length of the aerated Lemont and red rice roots (Fig. 3.5A & B; Table 3.1). In the first 10 mm behind the tips of these roots, little aerenchyma gas space was present. Therefore, along this portion of the root, internal gas phase oxygen diffusion to the growing tissues in the root tip would have to occur through non-lysigenous intercellular gas spaces (Kang *et al.* 1998).

In roots from the hypoxia-1.0 cm treatments, the most active cell collapse occurred between 4 and 19 mm behind the root tip (Fig. 3.6A & B). This is consistent with gas space development in primary rice roots that were generated from presoaked caryopses (Kawai *et al.* 1998). Between 19 and 36 mm behind the root tip, cortex gas space increased more gradually to a maximum of about 90% in both Lemont and red rice. Between 60 mm and sections that were nearest to the seed, mean cortex gas space declined to 79% of the maximum in Lemont and 83% of the maximum in red rice. Near-maximum amounts of cortex gas space were therefore found along over three-quarters of the length of the roots from the hypoxia-1.0 cm treatments (Fig. 3.6A & B; Table 3.1). The transport of oxygen to actively dividing and elongating cells would be enhanced in these roots because aerenchyma gas spaces began to form 1 to 4 mm behind the root tip.

In Lemont roots from the hypoxia-4.5 cm treatment, mean cortex gas space increased to a maximum of 39% along about three quarters of the length of the root and then decreased by 26% in sections closest to the seed (Fig. 3.6A). In red rice roots from the hypoxia-4.5 cm treatment, cortex gas space increased to a maximum of 55% along the youngest half of the root and then decreased by 49% along the remaining half of the root (Fig. 3.6B). Even though gas spaces were present within 1 mm of the root tip in these roots from the hypoxia-4.5 cm treatment, oxygen transport to the growing tissues would be inhibited because of the relatively small amount of cortex gas space further behind the root tip and because shoots were not in contact with the air.

Hypoxia did not seem to have a large effect upon the initial rate of cortex cell collapse to form aerenchyma as long as oxygen transport from the shoot to the root was possible. Cortex gas space resulting from cell collapse was first noticeable in tissues that were 9 to 15 h-old. The

initial increase in cortex gas space occurring between the tissue ages of about 10 and 45 h was similar among roots from the aerated-1.0 cm, aerated-4.5 cm (Fig. 3.5C & D) and hypoxia-1.0 cm treatments (Fig. 3.6C & D). Differences in the spatial distribution of gas space among roots from these treatments seem to be due to differences in root elongation rates. Roots from the hypoxia-1.0 cm treatment elongated more slowly than aerated roots. Therefore, the distances between the root tip and 10 h-old tissues and between 10 h-old and 45 h-old tissues were shorter in roots from the hypoxia-1.0 cm treatment. Consequently, aerenchyma formation occurred closer to the root tip and over a shorter distance in roots from the hypoxia-1.0 cm treatment. Roots from the hypoxia-1.0 cm treatment had a larger maximum amount of cortex gas space than aerated roots because gas space formation continued in tissues that were older than 45 h in the roots from the hypoxia-1.0 cm treatment. In aerated roots, mean cortex gas space fluctuated in tissues older than 45 h but never increased beyond the maximum. The close proximity of older aerated tissues to the seed may have influenced the amount of gas space formed.

In the roots from the hypoxia-4.5 cm treatment, a relatively slow increase in cortex gas space formation continued as tissues approached an age of 85 or more hours (Fig. 3.6C & D). Nevertheless, the amount of cortex gas space in roots from the hypoxia-4.5 cm treatment never approached that of roots in the other treatments. The smaller amounts of cortex gas space in the oldest tissues from the hypoxia-4.5 cm treatment were likely due to the nearness of the tissues to the seed as well as the inhibition of cell collapse.

In conclusion, when oxygen transport from the shoot was possible, hypoxic treatment resulted in a root anatomy that would allow more efficient transport of oxygen to the root tip than the root anatomy of aerated seedlings. When oxygen transport from the shoot was prevented, hypoxia drastically inhibited the amount of root aerenchyma and the rate of cortex cell collapse to form root aerenchyma. No other studies have demonstrated how hypoxia affects aerenchyma development in completely submerged rice seedlings. Further exploration of this topic could foster a better understanding of the regulation and mechanism of lysigenous aerenchyma formation at the cellular level and also of factors that determine the success of seedling establishment in the field.

Cortex cell arrangement The cortex cell arrangement in transverse sections taken close to the tip of primary rice roots (Fig. 3.4D & E) resembled the cubic radial packing in the C1(b)-type cortex defined by Justin and Armstrong (1987). In fact, the cortex of rice roots studied by Justin and Armstrong (1987) typified this category. In transverse sections of the primary root that displayed this cortex arrangement, we generally observed four cells in each radial file that extended between the sclerified outer cortex layer and the endodermis whereas Kawai *et al.* (1998) reported seven cells in each radial file. We counted 10 or more cells per radial file in some of the transverse sections of adventitious rice roots that were presented in the figures of Justin and Armstrong (1987) and Justin and Armstrong (1991). The cortex cell arrangement in transverse sections taken close to the seed (Fig. 3.4 A & B) somewhat resembled the M4(b)-type cortex defined by Justin and Armstrong (1987) in which hexagonal packing of cells occurred in the outer cortex layers while cubic radial packing occurred in the inner cortex. The authors placed some transverse sections of corn roots in this category. We observed that as distance from the seed increased, cells within the cortex gradually decreased in number and became more organized into a cubic radial cell arrangement. Justin and Armstrong (1987) similarly noted that

in some species like corn, the root cortex graded from one type to another along the length of the root but they did not report this for rice. Aerenchyma rarely forms when cells are packed hexagonally (Justin and Armstrong 1987). This may explain why cortex gas space was smaller and cells in the outer cortex were un-collapsed in many transverse sections taken close to the seed. Transverse sections of primary rice roots presented in the figures of Kawai *et al.* (1998) had, by our inspection, cortex arrangements that were characteristic of the variability in cortex arrangement that we observed.

Justin and Armstrong (1987) reason that hexagonal cell packing confers more stability and strength than the cubic packing and may be favored when there are mechanical and bending stresses. The presence of hexagonal cell packing in the outer cortex of primary rice roots that are just emerging from the seed supports their hypothesis. During germination and seedling establishment, the primary root of rice penetrates the tough pericarp, bends during gravitropic orientation and penetrates the soil. Hexagonal cell packing with fewer gas spaces may also facilitate the symplastic transport of seed resources to root tissues.

Shoots

The rice coleoptile is thought to provide a path for the diffusion of oxygen to the shoot meristem when a seedling germinates under oxygen-depleted conditions (Kordan 1974a; Takahashi 1978; Yamauchi *et al.* 1994; Yamauchi & Biswas 1997). The fact that coleoptile and primary leaf lengths responded similarly to submergence and hypoxia (Fig. 3.3) suggests that the two structures may have similar functions. Under anoxic conditions, only the coleoptile elongates (Öpik 1973; Ishizawa *et al.* 1999). Our results suggest that during seedling establishment in hypoxic environments, the primary leaf would acquire and transport oxygen better than the coleoptile because it is longer and has a greater amount of gas space.

Primary leaves In Lemont and red rice, primary leaves from the hypoxia-1.0 cm treatment had a larger transverse area, a greater number of gas space cavities and a greater amount gas space than primary leaves from the aerated-1.0 and aerated-4.5 cm treatments (Fig. 3.7). These differences are likely due to both treatment and age differences. Little primary leaf elongation occurred in the hypoxia-1.0 cm treatment after 118 h, the time that the aerated seedlings were harvested, but aerenchyma tissue within leaves from the hypoxia-1.0 cm treatment had 118 h longer to mature.

Primary leaves from the hypoxia-1.0 cm treatment also had a larger transverse area, more gas space cavities and more gas space than primary leaves from the hypoxia-4.5 cm treatment. These differences are due to treatment differences only. The relatively small transverse areas of primary leaves from the hypoxia-4.5 cm treatment suggest that horizontal expansion of the primary leaves was inhibited when seedlings were completely submerged in hypoxic water. This idea is further supported by fact that in transverse sections, coleoptiles were never split open in the hypoxia-4.5 cm treatment and always split open in the hypoxia-1.0 cm treatment. Even submergence in aerated water seems to have reduced primary leaf expansion given that, in both Lemont and red rice, transverse areas were smaller in the aerated-4.5 cm treatment compared to the aerated-1.0 cm treatment. Although the mean primary leaf lengths and the mean transverse areas of the leaves seemed to have opposite responses to treatment, there was not a strong negative correlation between these values when all data was pooled (data not shown).

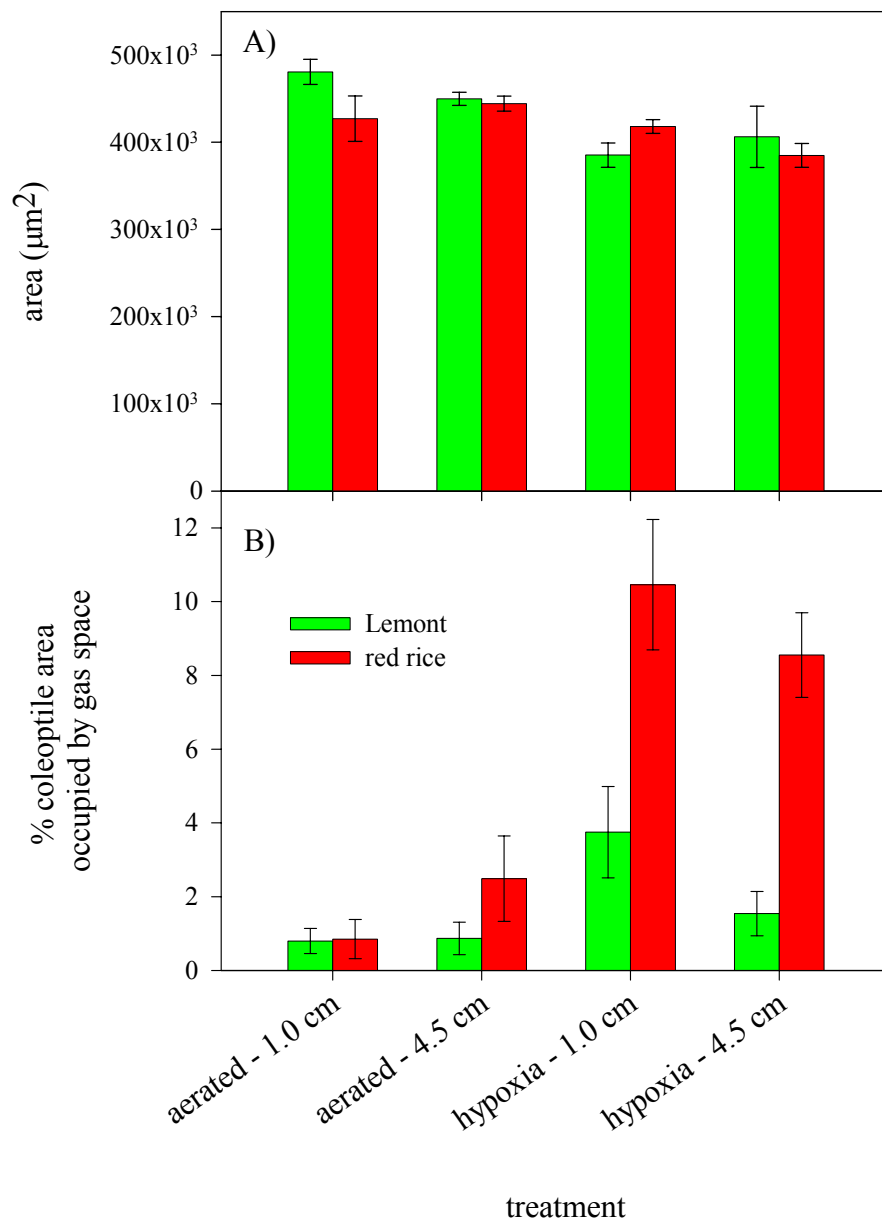


Figure 3.9 Area (A) and percent of area occupied by gas space (B) for transverse sections of coleoptiles taken between two and three mm above the coleoptile node in Lemont and red rice seedlings grown in aerated and hypoxic water. Germinated seeds were positioned at either 1.0 cm or 4.5 cm below the water surface so that as seedlings grew, leaves either emerged from the water or remained submerged. Values are means (± 1 S.E.) of four replicate coleoptile cross sections taken from four different seedlings.

Leaves from the hypoxia-1.0 cm treatments had a greater number of gas space cavities than leaves from other treatments (Fig. 3.7B). When viewing the transverse leaf sections, the additional gas cavities were located at the outer edges of the leaf and were small relative to the other gas cavities within the leaf (Fig. 3.1). Therefore, the transverse area of the additional gas cavities made only a small contribution to the total transverse area of gas space in the hypoxia-1.0 cm leaves. Leaves from the hypoxia-1.0 cm treatment had the largest amount of gas space (Fig. 3.7C) mainly because individual gas cavities were larger in this treatment. Red rice had a slightly larger mean amount of gas space than Lemont in transverse sections of leaves from all treatments except hypoxia-1.0 cm. However, this difference does not appear to be significant.

To our knowledge, this study is the first to report the effects of submergence and hypoxia on aerenchyma within primary leaves of rice seedlings. Further knowledge of how hypoxia affects the process of aerenchyma gas space formation in the primary leaf could be gained by examining serial transverse and longitudinal sections taken along the length of this organ. This approach might expose greater differences, if any exist, between rice strains.

Coleoptiles Coleoptile elongation can be attributed in large part to cell elongation (Fig. 3.8). The various treatments did not have a large effect on the transverse area of coleoptiles. Variability in coleoptile areas was likely due to a several factors such as normal variability among seedlings, differences in the amount of cell division and elongation among treatments, and differences in the amount of compaction that occurred due to leaf expansion.

In Lemont, effects of the various treatments on the amount of gas space were similar between the coleoptile (Fig. 3.9) and primary leaves (Fig. 3.7); hypoxia increased the mean amount of gas space when the shoots were emergent but not when the shoots were submerged. In aerated red rice coleoptiles, submergence resulted in some increase in the mean amount of gas space (Fig. 3.9). But, red rice coleoptiles from the hypoxia-1.0 cm and hypoxia-4.5 cm treatments had a much larger amount of gas space than Lemont and red rice coleoptiles from all other treatments. Differences in the amount gas space between transverse sections of Lemont and red coleoptiles may reflect differences at the cellular level. For example, red rice coleoptile cells may display greater sensitivity to hypoxia or to hormones, like ethylene, that may stimulate coleoptile gas space formation under hypoxic conditions. Gas space formation in the coleoptile may also be related to the process of senescence that was probably more progressed in the older coleoptiles from the hypoxia treatment. Although Inada *et al.* (1998a, b) did not observe coleoptile gas spaces, they demonstrated that an orderly sequence of cellular senescence near the tip of the coleoptile begins after the forth day of growth and is complete by the seventh day. We did not observe any external indications of tissue death in Lemont or red rice coleoptiles however cells within red rice coleoptiles from the hypoxia treatments may have been in a more advanced stage of senescence than cells within Lemont coleoptiles.

This study is also the first to report the presence of aerenchyma in coleoptiles. As with primary leaves, an investigation of serial transverse and longitudinal sections taken along the length of the coleoptile would increase knowledge about aerenchyma gas space formation in the coleoptile. This approach may also help explain differences in aerenchyma between Lemont and red rice coleoptiles. Further study is also necessary to determine if aerenchyma in the coleoptile facilitates internal gas transport or has other specialized functions.

CHAPTER 4. SUMMARY OF STUDY

Aerenchyma, a specialized tissue with abundant, interconnected gas spaces, is common in the roots and shoots of many emergent wetland plants (Smirnoff & Crawford 1983; Justin & Armstrong 1987; Schussler & Longstreth 1996). Oxygen transport through aerenchyma is believed to enable plant growth in hypoxic, flooded environments by augmenting aerobic respiration in roots, rhizomes, and submerged leaves. Nevertheless, few studies have demonstrated a correlation between oxygen transport through aerenchyma and increased rates of growth. The importance of internal oxygen transport to root elongation and aerenchyma development in rice seedlings was demonstrated by the experiments presented herein. In each experiment, germinating seeds of two strains of rice, Lemont and red rice, were positioned below the surface of aerated and hypoxic water. The depth at which the seeds were positioned controlled the amount of time it took for leaves to emerge from the water. Previous research demonstrated internal oxygen transport in rice plants (Barber *et al.* 1962; Webb & Armstrong 1983; Armstrong & Webb 1985). We therefore presumed that leaf contact with the atmosphere established an oxygen concentration gradient within the seedling that drove oxygen diffusion towards submerged tissues. This study is notably unique in two ways. First, the experiments quantitatively demonstrated how root elongation rates change when leaves of submerged rice seedlings emerge into the air. Secondly, the research revealed how hypoxia affected aerenchyma formation when oxygen transport was prevented by complete submergence in hypoxic water.

Elongation rates of primary roots were similar among aerated seedlings positioned at different depths (Fig. 2.4). Root elongation rates did not change after leaves emerged from aerated water (Figs. 2.4 & 2.7). Dissolved oxygen in the aerated water was presumably sufficient to support the maximum rates of root elongation that the aerated seedlings displayed. When seedlings were started at 1.0 cm and 2.5 cm below the surface of hypoxic water, root elongation rates increased within the same interval of time as leaf emergence (Figs. 2.5 & 2.6). These results suggested that diffusion-driven, internal oxygen transport can enhance seedling growth provided that a portion of the leaf is contacting the atmosphere and that remaining portions of the seedling lack oxygen. A deeper submergence depth may compromise the benefit of oxygen transport to seedling growth once leaves emerge from the water. After leaves emerged from 4.5 cm of overlying hypoxic water, a greater distance for diffusion, a small volume of gas space, oxygen consumption by submerged tissues, and diffusion of oxygen out of the seedling may have collectively inhibited internal oxygen diffusion to the root tip and hence prevented faster root elongation following leaf emergence (Figs. 2.5 & 2.6).

The root anatomy of seedlings that grew in hypoxic water with emergent leaves, would seem to allow the most efficient transport of oxygen to the root tip. In these seedlings, near-maximum amounts of aerenchyma gas space were found along over three-quarters of the length of the roots (Fig. 3.6A & B). And, aerenchyma gas spaces within 4 mm of the root tips could enhance the delivery of oxygen to actively dividing and elongating cells. In aerated roots, the process of gas space formation, progressing back from the root tip, occupied over half the length of the roots (Fig. 3.5A & B). Because little aerenchyma gas space was present in the first 10 mm behind the aerated root tips, internal, gas phase oxygen diffusion to the actively growing tissues would have to occur through small, non-lysigenous intercellular gas spaces within this portion of the root (Kang *et al.* 1998). In seedlings that grew completely submerged in hypoxic water, oxygen

transport to the root tip would be inhibited because a relatively small amount of gas space developed throughout the roots and because shoots were not in contact with the air (Fig. 3.6A & B).

Hypoxia did not seem to affect the initial rate of cortex cell collapse to form aerenchyma as long as oxygen transport from the shoot to the root was possible. The initial increase in aerenchyma gas space, occurring between the tissue ages of about 10 and 45 h, was similar among aerated roots and roots of emergent seedlings that grew in hypoxic water (Figs. 3.5 & 3.6). Differences in the spatial distribution of gas space among roots from these treatments seem to be due to differences in root elongation rates. Roots of emergent seedlings that grew in hypoxic water elongated more slowly than aerated roots (Appendix 1). Thus, the distances between the root tip and 10 h-old tissues and between 10 h-old and 45 h-old tissues were shorter in roots from emergent seedlings that grew in hypoxic water than in aerated roots. Aerenchyma formation therefore occurred closer to the root tip and over a shorter distance in roots of emergent seedlings that grew in hypoxic water. Roots from emergent seedlings that grew in hypoxic water had a larger maximum amount of aerenchyma gas space than aerated roots because gas space formation continued in tissues that were older than 45 h (Fig. 3.6C & D). In aerated roots, the amount of gas space fluctuated in tissues older than 45 h but never increased above the maximum (Fig. 3.5C & D). The close proximity of older aerated tissues to the seed may have influenced the amount of gas space formed. When oxygen transport from the shoot was prevented, hypoxia inhibited both root elongation and cell collapse. In the relatively short roots of seedlings that grew completely submerged in hypoxic water, a slow increase in gas space formation continued as tissues approached an age of 85 or more hours. Nevertheless, the amount of gas space in these roots never reached that of roots from the other treatments (Fig. 3.6C & D).

This study is the first to describe the effects of hypoxia on aerenchyma within coleoptiles and primary leaves of submerged and emergent rice seedlings. Additionally, aerenchyma in coleoptiles has not been reported until now. Measurements of transverse shoot sections taken between 2 and 3 mm above the seed revealed that 1) red rice coleoptiles grown in hypoxic water had 3 to 12 times more gas space than aerated coleoptiles (Fig. 3.9) and 2) the amount of gas space in primary leaves was two to five times greater in emergent seedlings that grew in hypoxic water than in aerated seedlings and completely submerged seedlings that grew in hypoxic water (Fig. 3.7). As with root aerenchyma, our data suggest that leaf aerenchyma development is inhibited by complete submergence in hypoxic water. Further knowledge of how hypoxia affects the process of aerenchyma gas space formation in the coleoptile and primary leaf could be gained by using serial transverse and longitudinal sections to examine the anatomy of these structures. This approach might illuminate differences between rice strains in shoot aerenchyma.

The cylindrical rice coleoptile is believed to function as conduit for the external transport of oxygen when seeds germinate in saturated, oxygen-depleted soil (Kordan 1974a; Takahashi 1978; Yamauchi *et al.* 1994; Yamauchi & Biswas 1997). However, the ability of the rice coleoptile to transport oxygen has not been tested experimentally. In wheat seedlings, the coleoptile actually impedes the movement of oxygen from the shoot to the root tip (Thomson & Armstrong 1990). Additional research is necessary to determine if aerenchyma in the coleoptile facilitates internal gas transport or serves other functions. In our experiments, coleoptiles did not elongate into the air except when seedlings were started at 1.0 cm below the water surface. The

primary leaf was usually the first structure to emerge from the water. Similar elongation responses of coleoptiles and primary leaves to submergence and hypoxia (Fig. 3.3) suggest that the two structures may have similar functions. Only the coleoptile will elongate under anoxic conditions (Öpik 1973; Ishizawa *et al.* 1999). But when conditions are hypoxic, our results indicate that the primary leaf would acquire and transport oxygen better than the coleoptile because it grows longer and has a greater amount of gas space. A bladeless primary leaf with aerenchyma may be an adaptation that works in concert with the coleoptile to facilitate rice seedling establishment in flooded soils.

Lemont and red rice seedlings between the germination and first leaf stages of development demonstrated the capacity for internal oxygen transport. Differences in elongation and aerenchyma development between Lemont and red rice roots were generally small compared to differences between aerated roots and roots from the hypoxia treatments. The relative importance of internal oxygen transport to Lemont and red rice seedlings must be considered in the context of each rice strain's response to factors like submergence, oxygen concentration and light. Reminiscent of the robust growth that red rice biotypes exhibit in the field, red rice emerged from aerated water sooner than Lemont (Table 2.1) and the root and leaf lengths of red rice became increasingly larger than those of Lemont during 120 h of growth in aerated water (Fig. 2.4 & 2.7). The presence or absence of light did not affect the average time of leaf emergence from aerated water in Lemont or red rice (Fig. 2.7, Table 2.1). Prior to leaf emergence from hypoxic water, Lemont and red rice seedlings positioned at a depth of 2.5 cm had similarly slow root elongation rates, both in the presence and absence of a 12 h photoperiod (Fig. 2.8). Leaf emergence from 1.0 and 2.5 cm of overlying hypoxic water was associated with faster root elongation in both Lemont and red rice (Figs. 2.5, 2.6 & 2.8). With continuous darkness, Lemont seedlings in the single depth experiment had a "head start" because the average leaf emergence time was earlier in Lemont than in red rice (Table 2.2). This may explain why the average leaf and root lengths of red rice only exceeded those of Lemont after 240 h (Fig. 2.8A & B). A 12 h photoperiod delayed leaf emergence in Lemont (Table 2.2). Consequently, red rice had an earlier average emergence time than Lemont and between the time of leaf emergence and the end of the experiment, red rice continued to have larger average leaf and root lengths than Lemont (Fig. 2.8C & D). These data suggest that the relative time of leaf emergence, affected by submergence depth and the availability of light, may be important to the competitive interactions among seedlings that are becoming established in flooded fields. However, by inhibiting aerenchyma gas space development and oxygen transport, water that is too deep and depleted of oxygen may delay or even prohibit the establishment of both Lemont and red rice seedlings in the field.

LITERATURE CITED

- Armstrong W. (1971) Radial oxygen losses from intact rice roots as affected by distance from the apex, respiration and waterlogging. *Physiol. Plant.* **25**, 192-197.
- Armstrong W. & Webb T. (1985) A critical oxygen pressure for root extension in rice. *Journal of Experimental Botany* **36**, 1573-1582.
- Atwell B.J., Waters I. & Greenway H. (1982) The effect of oxygen and turbulence on elongation of coleoptiles of submergence-tolerant and -intolerant rice cultivars. *Journal of Experimental Botany* **33**, 1030-1044.
- Aulakh M.S., Wassmann R., Rennenberg H. & Fink S. (2000) Pattern and amount of aerenchyma relate to variable methane transport capacity of different rice cultivars. *Plant biol.* **2**, 182-194.
- Barber D.A., Ebert M. & Evans N.T.S. (1962) The movement of ^{15}O through barley and rice plants. *Journal of Experimental Botany* **13**, 397-403.
- Barclay A.M. & Crawford R.M.M. (1982) Plant growth and survival under strict anaerobiosis. *Journal of Experimental Botany* **33**, 541-549.
- Butterbach-Bahl K., Papen H. & Rennenberg H. (1997) Impact of gas transport through rice cultivars on methane emission from rice paddy fields. *Plant, Cell and Environment* **20**, 1175-1183.
- Butterbach-Bahl K., Papen H. & Rennenberg H. (2000) Scanning electron microscopy analysis of the aerenchyma in two rice cultivars. *Phyton (Horn, Austria)* **40**, 43-55.
- Campbell R. & Drew M.C. (1983) Electron microscopy of gas space (aerenchyma) formation in adventitious roots of *Zea mays* L. subjected to oxygen shortage. *Planta* **157**, 350-357.
- Clark L.H. & Harris W.H. (1981) Observations on the root anatomy of rice (*Oryza sativa* L.). *American Journal of Botany* **68**, 154-161.
- Colmer T.D., Gibberd M.R., Wiengweera A. & Tinh T.K. (1998) The barrier to radial oxygen loss from roots of rice (*Oryza sativa* L.) is induced by growth in stagnant solution. *Journal of Experimental Botany* **49**, 1431-1436.
- Das D.K. & Jat R.L. (1977) Influence of three soil-water regimes on root porosity and growth of four rice varieties. *Agronomy Journal* **69**, 197-200.
- Datta S.K. & Banerji B. (1974) Anatomical variations of flood-resistant and deep-water rices under field and deep-water conditions. *Phytomorphology* **24** 164-174.

Diarra A., Smith Jr. R.J. & Talbert R.E. (1985) Growth and morphological characteristics of red rice (*Oryza sativa*) biotypes. *Weed Science* **33**, 310-314.

Dunand R.T. (1998) Growth and development of the rice plant. In: Louisiana Rice Production Handbook. pp. 12-20. Louisiana State University Agricultural Center, Louisiana Cooperative Extension Service. Pub. 2321. Rev. 11/98.

Esau, K. (1977) Anatomy of seed plants, 2nd Edition. John Wiley & Sons, Inc., New York.

Gambrell R.P., DeLaune R.D. & Patrick W.H. Jr. (1991) Redox processes in soils following oxygen depletion. In: Plant Life Under Oxygen Deprivation: Ecology, Physiology and Biochemistry (eds Jackson M.B., D.D. Davies & H. Lambers), pp. 101-117. SPB Academic Publishing bv, The Hague, The Netherlands.

Gibberd M.R., Colmer T.D. & Cocks P.S. (1999) Root porosity and oxygen movement in waterlogging-tolerant *Trifolium tomentosum* and -intolerant *Trifolium glomeratum*. *Plant, Cell and Environment* **22**, 1161-1168.

Guglielminetti L., Perata P. & Alpi A. (1995) Effect of anoxia on carbohydrate metabolism in rice seedlings. *Plant Physiol.* **108**, 735-741.

He J.B., Bögemann G.M., van de Steeg H.M., Rijnders J.G.H.M., Voeseek L.A.C.J. & Blom C.W.P.M. (1999) Survival tactics of *Ranunculus* species in river floodplains. *Oecologia* **118**, 1-8.

Higuchi, T. (1982) Gaseous CO₂ transport through the aerenchyma and intercellular spaces in relation to the uptake of CO₂ by rice roots. *Soil Sci. Plant Nutr.* **28**, 491-497.

Higuchi T., Yoda K. & Tensho K. (1984) Further evidence for gaseous CO₂ transport in relation to root uptake of CO₂ in rice plant. *Soil Sci. Plant Nutr.* **30**, 125-136.

Iijima M., Oribe Y., Horibe Y. & Kono Y. (1998) Time lapse analysis of root elongation rates of rice and sorghum during the day and night. *Annals of Botany* **81**, 603-607.

Inada N., Sakai A., Kuroiwa H. & Kuroiwa T. (1998) Three-dimensional analysis of the senescence program in rice (*Oryza sativa* L.) coleoptiles: Investigations by fluorescence microscopy and electron microscopy. *Planta* **206**, 585-597.

Inada N., Sakai A., Kuroiwa H. & Kuroiwa T. (1998) Three-dimensional analysis of the senescence program in rice (*Oryza sativa* L.) coleoptiles: Investigations of tissues and cells by fluorescence microscopy. *Planta* **205**, 153-164.

Ishizawa K. & Esashi Y. (1983) Cooperation of ethylene and auxin in the growth regulation of rice coleoptile segments. *Journal of Experimental Botany* **34**, 74-82.

Ishizawa K. & Esashi Y. (1984) Gaseous factors involved in the enhanced elongation of rice coleoptiles under water. *Plant, Cell and Environment* **7**, 239-245.

- Ishizawa K., Murakami S., Kawakami Y. & Kuramochi H. (1999) Growth and energy status of arrowhead tubers, pondweed turions and rice seedlings under anoxic conditions. *Plant, Cell and Environment* **22**, 505-514.
- Jackson M.B. (1985) Ethylene and responses of plants to soil waterlogging and submergence. *Ann Rev. Plant Physiol.* **36**, 145-174.
- Jackson M.B. & Armstrong W. (1999) Formation of aerenchyma and the processes of plant ventilation in relation to soil flooding and submergence. *Plant Biol.* **1**, 274-287.
- Jackson M.B., Fenning T.M., Drew M.C., Saker L.R. (1985a) Stimulation of ethylene production and gas-space (aerenchyma) formation in adventitious roots of *Zea mays* L. by small partial pressures of oxygen. *Planta* **165**, 486-492.
- Jackson M.B., Fenning T.M. & Jenkins W. (1985b) Aerenchyma (gas-space) formation in adventitious roots of rice (*Oryza sativa* L.) is not controlled by ethylene or small partial pressures of oxygen. *Journal of Experimental Botany* **36**, 1566-1572.
- John C.D. (1977) The structure of rice roots grown in aerobic and anaerobic environments. *Plant and Soil* **47**, 269-274.
- Jordan D. and Sanders D.E. (1998) Pest management. In: Louisiana Rice Production Handbook. pp. 37-41. Louisiana State University Agricultural Center, Louisiana Cooperative Extension Service. Pub. 2321. Rev. 11/98.
- Justin S.H.F.W. & Armstrong W. (1987) The anatomical characteristics of roots and plant response to soil flooding. *New Phytol.* **106**, 465-495.
- Justin S.H.F.W. & Armstrong W. (1991) Evidence for the involvement of ethene in aerenchyma formation in adventitious roots of rice (*Oryza sativa* L.). *New Phytol.* **118**, 49-62.
- Kang S-Y, Wada T. & Choi K-S. (1998) Development of schizogenous and lysigenous aerenchyma in rice root. *Korean J. Crop Sci.* **43**, 77-82.
- Katayama T. (1960) Studies on the intercellular spaces in rice. I. *Proceedings of the Crop Science Society of Japan* **29**, 229-233.
- Katsura N. & Suge H. (1979) Does ethylene induce elongation of the rice coleoptile through auxin. *Plant and Cell Physiol.* **20**, 1147-1150.
- Kaufman P.B. (1959) Development of the shoot of *Oryza sativa* L. – II. Leaf histogenesis. *Phytomorphology* **9**, 277-311.
- Kawai M., Samarajeewa P.K., Barrero R.A., Nishiguchi M. & Uchimiya H. (1998) Cellular dissection of the degradation pattern of cortical cell death during aerenchyma formation of rice roots. *Planta* **204**, 277-287.

- Kennedy R.A., Barrett S.C.H., Zee D.V. & Rumpho M.E. (1980) Germination and seedling growth under anaerobic conditions in *Echinochloa crus-galli* (barnyard grass). *Plant, Cell and Environment* **3**, 243-248.
- Kludze H.K., DeLaune R.D. & Patrick Jr. W.H. (1994) A colorimetric method for assaying dissolved oxygen loss from container-grown rice roots. *Agron. J.* **86**, 483-487.
- Končalová H. (1990) Anatomical adaptations to waterlogging in roots of wetland graminoids: limitations and drawbacks. *Aquatic Botany* **38**, 127-134.
- Kordan H.A. (1972) Rice seedlings germinated in water with normal and impeded environmental gas exchange. *Journal of Applied Ecology* **9**, 527-533.
- Kordan H.A. (1974a) Patterns of shoot and root growth in rice seedlings germinating under water. *Journal of Applied Ecology* **11**, 685-690.
- Kordan H.A. (1974b) The rice shoot in relation to oxygen supply and root growth in seedlings germinating under water. *New Phytol.* **73**, 695-697.
- Kordan H.A. (1975) Relationship between oxygen availability and transverse and vertical shoot geotropisms during germination of submerged rice seedlings. *Annals of Botany* **39**, 249-56.
- Kordan H.A. (1976) Oxygen as an environmental factor in influencing normal morphogenetic development in germinating rice seedlings. *Journal of Experimental Botany* **27**, 947-952.
- Kordan H.A. (1977a) Coleoptile emergence in rice seedlings in different oxygen environments. *Annals of Botany* **41**, 1205-1209.
- Kordan H.A. (1977b) Latent effect of anaerobiosis on root growth in germinating rice seedlings. *Plant Science Letters* **9**, 53-56.
- Ku H.S., Suge H., Rappaport L. & Pratt H.K. (1970) Stimulation of rice coleoptile growth by ethylene. *Planta* **90**, 333-339.
- Kwon S.L., Smith Jr. R.J. & Talbert R.E. (1991a) Interference durations of red rice (*Oryza sativa*) densities in rice (*O. sativa*). *Weed Science* **39**, 363-368.
- Kwon S.L., Smith Jr. R.J. & Talbert R.E. (1991b) Interference of red rice (*Oryza sativa*) densities in rice (*O. sativa*). *Weed Science* **39**, 169-174.
- Kwon S.L., Smith Jr. R.J. & Talbert R.E. (1992) Comparative growth and development of red rice (*Oryza sativa*) and rice (*O. sativa*). *Weed Science* **40**, 57-62.
- LSU Ag Center Research and Extension, Louisiana State University Agriculture Center. Pub. 2270, 10/02 Rev.

- Matsukura C., Kawai M., Toyofuku K., Barrero R.A., Uchimiya H., & Yamaguchi J. (2000) Transverse vein differentiation associated with gas space formation – fate of the middle cell layer in leaf sheath development of rice. *Annals of Botany* **85**, 19-27.
- Ohwaki Y. (1967) Growth of rice coleoptiles in relation to oxygen concentrations. *Sci. Rep. Tohoku Univ. Ser. IV (Biol.)* **33**, 1-5.
- Öpik H. (1973) Effect of anaerobiosis on respiratory rate, cytochrome oxidase activity and mitochondrial structures in coleoptiles of rice (*Oryza sativa* L.). *J. Cell Sci.* **12**, 725-739.
- Perata, P., Loreti E., Guglielminetti L. & Alpi A. (1998) Carbohydrate metabolism and anoxia tolerance in cereal grains. *Acta Bot. Neerl.* **47**, 269-283.
- Pfister-Sieber M. & Brändle R. (1994) Aspects of plant behaviour under anoxia and post-anoxia. *Proceedings of the Royal Society of Edinburgh* **102B**, 313-324.
- Ponnamperuma F.N. (1972) The chemistry of submerged soils. *Adv. Agron.* **24**, 29-96.
- Prade K. & Trollenier G. (1990) Denitrification in the rhizosphere of plants with inherently different aerenchyma formation: wheat (*Triticum aestivum*) and rice (*Oryza sativa*). *Biol Fertil Soils* **9**, 215-219.
- Ranson S.L. & Parija B. (1955) Experiments on growth in length of plant organs II. some effects of depressed oxygen concentrations. *Journal of Experimental Botany* **6**, 80-93.
- Raskin I. & Kende H. (1983a) How does deep water rice solve its aeration problem. *Plant Physiol.* **72**, 447-454.
- Raskin I. & Kende H. (1983b) Regulation of growth in rice seedlings. *Plant Growth Regul* **2**, 193-203.
- Raskin I. & Kende H. (1985) Mechanism of aeration in rice. *Science* **228**, 327-329.
- Samarajeewa P.K., Barrero R.A., Umeda-Hara C., Kawai M., & Uchimiya H. (1999) Cortical cell death, cell proliferation, macromolecular movements and *rTip1* expression pattern in roots of rice (*Oryza sativa* L.) under NaCl stress. *Planta* **207**, 354-361.
- Schussler E.E. & Longstreth D.J. (1996) Aerenchyma develops by cell lysis in roots and cell separation in leaf petioles in *Sagittaria lancifolia* (Alismataceae). *American Journal of Botany* **83**, 1266-1273.
- Setter T.L. & Ella E.S. (1994) Relationship between coleoptile elongation and alcoholic fermentation in rice exposed to anoxia. I. Importance of treatment conditions and different tissues. *Annals of Botany* **74**, 265-271.

Smirnoff N. and Crawford R.M.M. (1983) Variation in the structure and response to flooding of root aerenchyma in some wetland plants. *Ann. Bot.* **51**, 237-249.

Suge H. (1971) Stimulation of oat and rice mesocotyl growth by ethylene. *Plant and Cell Physiol.* **12**, 831-837.

Suge H. (1972) Mesocotyl elongation in *japonica* rice: Effect of high temperature pre-treatment and ethylene. *Plant and Cell Physiol.* **13**, 401-405.

Summers J.E. and Jackson M.B. (1994) Anaerobic conditions strongly promote extension by stems of overwintering tubers of *Potamogeton pectinatus* L. *Journal of Experimental Botany* **45**, 1309-1318.

Sylvester A.W., Parker-Clark V., & Murray G.A. (2001) Leaf shape and anatomy as indicators of phase change in the grasses: comparison of maize, rice, and bluegrass. *American Journal of Botany* **99**, 2157-2167.

Taiz L. & Zeiger E. (1998) Plant Physiology, Second Edition. Sinauer Associates, Inc., Sunderland Massachusetts.

Takahashi K. (1972) Absciscic acid as a stimulator for rice mesocotyl growth. *Nature New Biology* **238**, 92-93.

Takahashi K. (1973) Interaction between ethylene, absciscic acid and gibberellic acid in elongation of rice mesocotyl. *Planta* **109**, 363-364.

Takahashi N. (1978) Adaptive importance of mesocotyl and coleoptile growth in rice under different moisture regimes. *Aust. J. Plant Physiol.* **5**, 511-517.

Thompson C.J. & Armstrong W. (1990) The coleoptile sheath of wheat impedes oxygen transport to seminal roots. *Plant, Cell and Environment* **13**, 405-406.

Turner F.T., Chen C-C. & Bollich C. N. (1982) Coleoptile and mesocotyl lengths in semidwarf rice seedlings. *Crop Science* **22**, 43-46.

Turner F.T., Chen C-C. & McCauley G.N. (1981) Morphological development of rice seedlings in water at controlled oxygen levels. *Agronomy Journal* **73**, 566-570.

Ushimaru T., Shibasaka M. & Tsuji H. (1992) Development of the O₂⁻-detoxification system during adaptation to air of submerged rice seedlings. *Plant Cell Physiology* **33**, 1065-1071.

Vartapetian B.B., Andreeva I.N., Kozlova G.I. & Agapova L.P. (1977) Mitochondrial ultrastructure in roots of mesophyte and hydrophyte at anoxia and after glucose feeding. *Protoplasma* **91**, 243-256.

- Vartapetian B.B. & Jackson M.B. (1997) Plant adaptations to anaerobic stress. *Annals of Botany* **79**, 3-20.
- Vartapetyan B.B., Andreeva I.N. & Maslova I.P. (1969) Oxygen regime and ultrastructure of rice root cells. *Doklady Akademii Nauk (SSSR)* **189**, 183-186. (English translation)
- Visser E.J.W., Blom C.W.P.M. & Voesenek L.A.C.J. (1996) Flooding-induced adventitious rooting in *Rumex*: morphology and development in an ecological perspective. *Acta Bot. Neerl.* **45**, 17-28.
- Visser E.J.W., Nabben R.H.M., Blom C.W.P.M. & Voesenek L.A.C.J. (1997) Elongation of primary lateral roots and adventitious root during conditions of hypoxia and high ethylene concentrations. *Plant, Cell and Environment* **20**, 647-653.
- Voesenek L.A.C.J., van der Sman A.J.M., Harren F.J.M. & Blom C.W.P.M. (1992) An amalgamation between hormone physiology and plant ecology: A review on flooding resistance and ethylene. *J Plant Growth Regul* **11**, 171-188.
- Waters I., Armstrong W., Thomson C.J., Setter T.L., Adkins S., Gibbs J. & Greenway H. (1989) Diurnal changes in radial oxygen loss and ethanol metabolism in roots of submerged and non-submerged rice seedlings. *New Phytol.* **113**, 439-451.
- Watkin E.L.J., Thomson C.J. & Greenway H. (1998) Root development and aerenchyma formation in two wheat cultivars and one triticale cultivar grown in stagnant agar and aerated nutrient solution. *Annals of Botany* **81**, 349-354.
- Webb T. & Armstrong W. (1983) The effects of anoxia and carbohydrates on the growth and viability of rice, pea and pumpkin roots. *Journal of Experimental Botany* **34**, 579-603.
- Webb J. & Jackson M.B. (1986) A transmission and cryo-scanning electron microscopy study of the formation of aerenchyma (cortical gas-filled space) in adventitious roots of rice (*Oryza sativa*). *Journal of Experimental Botany* **37**, 832-841.
- Yamauchi M. & Biswas J.K. (1997) Rice cultivar difference in seedling establishment in flooded soil. *Plant and soil* **189**, 145-153.
- Yamauchi M., Herradura P.S. & Aguilar A.M. (1994) Genotype difference in rice postgermination growth under hypoxia. *Plant Science* **100**, 105-113.
- Yamauchi M. & Winn T. (1996) Seed physiology, production and technology: Rice seed vigor and seedling establishment in anaerobic soil. *Crop Sci.* **36**, 680-686.
- Zarra I. & Masuda Y. (1979) Growth and cell wall changes in rice coleoptiles growing under different conditions I. Changes in turgor pressure and cell wall polysaccharides during intact growth. *Plant and Cell Physiol.* **20**, 1117-1124.

APPENDIX 1. GROWTH OF SEEDLINGS USED FOR ANATOMICAL ANALYSIS

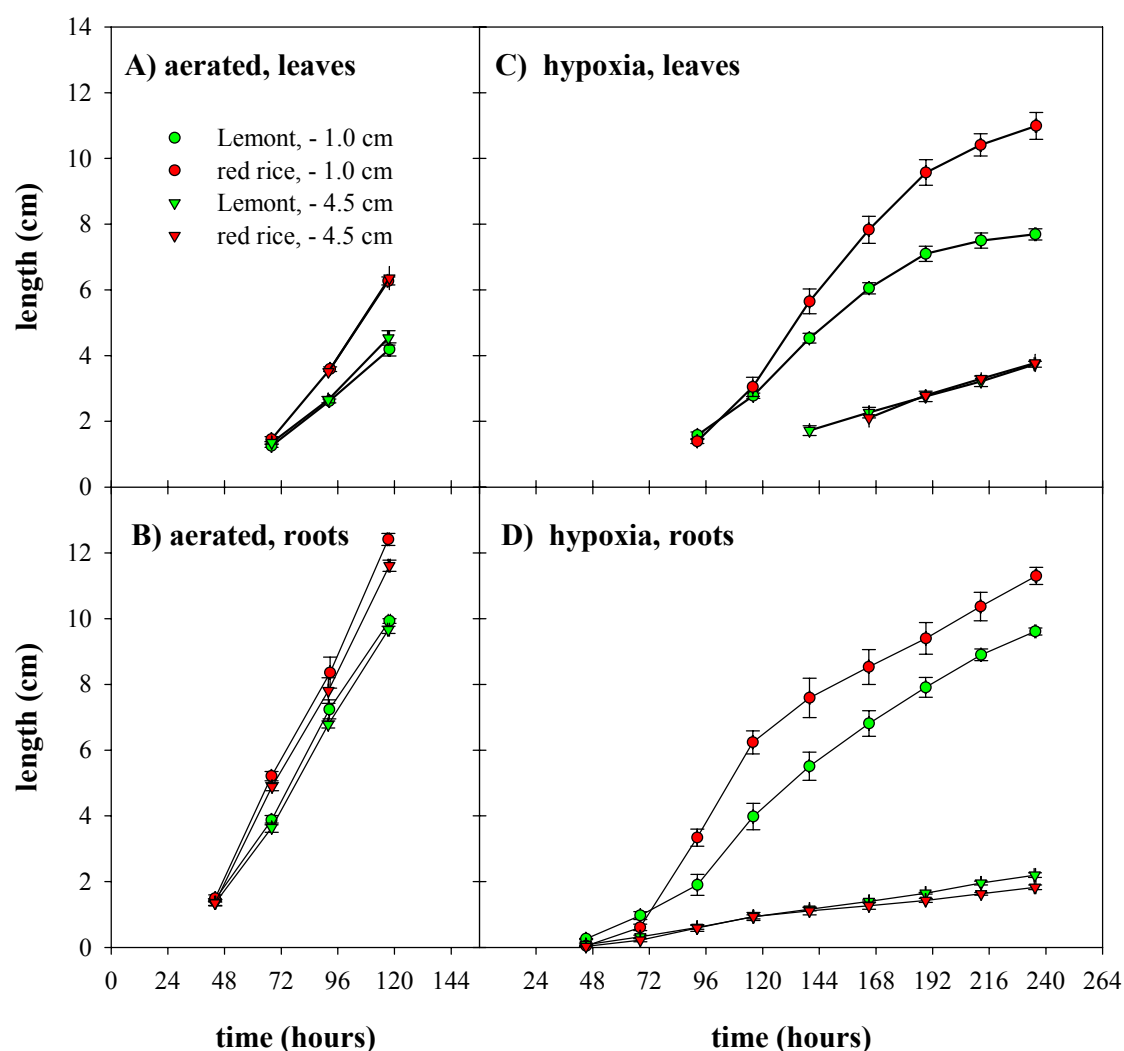
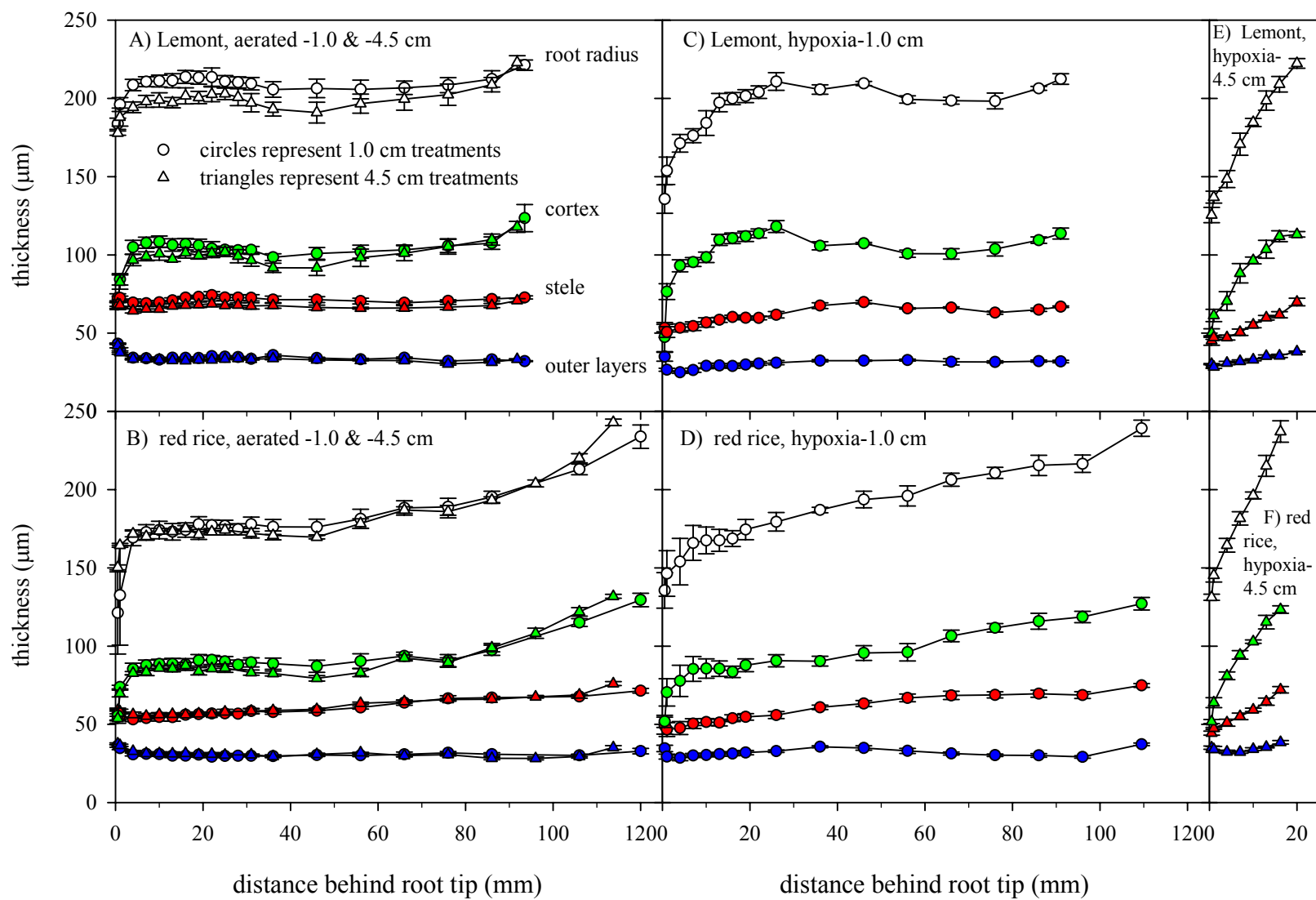


Figure A1.1 Leaf and root lengths of Lemont and red rice seedlings grown in aerated (A & B) and hypoxic (C & D) water. Germinated seeds were positioned 1.0 and 4.5 cm below the water surface so that seedlings were either emergent or completely submerged during the growth period. Values are mean lengths (± 1 S.E.) of 4 seedlings.

APPENDIX 2. ROOT RADII AND THICKNESSES OF THE CORTEX, STELE AND OUTER LAYERS

Figure A2.1 Root radius and thicknesses of the cortex, stele and outer layers in root cross sections presented as a function of distance behind the root tip. Roots were from Lemont and red rice seedlings grown in aerated and hypoxic water. Germinated seeds were positioned at either 1.0 cm (circles) or 4.5 cm (triangles) below the water surface so that as seedlings grew, leaves either emerged from the water or remained submerged. Curve labels in A apply to each frame. For both Lemont and red rice, curves representing the hypoxia-1.0 cm (C and D) and hypoxia-4.5 cm (E and F) treatments are presented in different frames for easier viewing. Values are means (± 1 S.E.) of four replicate root sections taken at the same distance behind the root tips of four roots.



VITA

Denise Marie D'Abundo was born July 11, 1969. She grew up in Marmora, a town located along the southern coast of New Jersey. Weekly fishing excursions in nearby estuaries and the ensuing Sunday dinners of crabs and spaghetti shared with her extended family instilled Denise's love of family, food and wetlands. While growing up, Denise developed interests in school, sports and music through studying fairly hard, participating on field hockey and track teams and playing the piano and trombone. After graduating from Ocean City High School in 1987, she attended Bucknell University in Lewisburg, Pennsylvania, for two years. Following a semester-long internship at the Smithsonian Environmental Research Center in Edgewater, Maryland, Denise transferred to Oberlin College in Oberlin, Ohio, where she earned a Bachelor of Arts degree in biology in 1992 and contently dabbled with studio art. Her Honors thesis at Oberlin College investigated the population genetics of *Vallisneria americana*, a submerged aquatic macrophyte that grows in Lake Erie. After a few carefree years, Denise moved to Baton Rouge, Louisiana, to begin a doctoral program in plant biology at Louisiana State University. The landscape, music, food, and culture of Louisiana appealed to Denise but at times contrasted with her northern upbringing. For motivation during her later years in graduate school, Denise pitted her intellectual and physical skills in a personal challenge to see which she could complete first, a dissertation or a marathon. She finished 26.2 miles in 2002. Denise's dissertation research on the physiology and anatomy of rice seedlings allowed her to balance interests in biology, wetland plants and aesthetically pleasing activities. After earning the degree of Doctor of Philosophy in 2003, Denise continued to seek challenging ways to meld her interests.